ABSTRACT

Title of dissertation: THE EMOTIONAL MODULATION OF THE STARTLE REFLEX IN 9-MONTH-OLD INFANTS

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The purpose of this research project was to investigate whether the startle reflex of 9-month-old infants can be modulated by emotional stimuli, as well as to examine the specific characteristics of infants' startle reactions in an emotion-modulated paradigm. Two studies were conducted to address these questions. In Study 1, 32 9-month-old infants viewed photographs of happy, neutral, and angry facial expressions. Infants' startle responses to acoustic probes during the presentation of the facial stimuli were recorded and compared across the three affective conditions. Autonomic and looking time data were also gathered in order to evaluate the contribution of other factors, such as attention, to the modulation of the startle reflex. The results of this study indicated a pattern of startle modulation opposite to that documented in adults. Infants demonstrated a potentiated startle reflex during the viewing of happy faces and an inhibited response

during the viewing of angry faces. Differences in heart period and looking time between the affective conditions suggested that these findings were driven, at least in part, by greater allocation of attentional resources to angry expressions.

To further examine the role of emotion in infants' startle modulation, an independent group of 25 9-month-old infants was tested in a second, modified emotion-modulated startle paradigm that involved the presentation of acoustic startle probes while infants were engaged in a pleasant game of peek-a-boo, an affectively neutral presentation of a spinning bingo wheel, and a mildly frustrating arm restraint episode. Autonomic and behavioral data were also gathered. As expected, the results revealed startle potentiation during the unpleasant condition and startle inhibition during the pleasant condition, indicating the existence of the emotion-modulated startle reflex in 9-month-old infants.

Results of both studies are discussed in terms of the role of emotion and attention in startle modulation, the maturation of the appetitive and defensive brain systems in infants, and the importance of establishing a rigorous and age-appropriate startle paradigm to foster the study of infants' emotionality. Suggestions for further studies utilizing such a paradigm to investigate different aspects of emotional reactivity in infancy are also proposed.

THE EMOTIONAL MODULATION OF THE STARTLE REFLEX

IN 9-MONTH-OLD INFANTS

by
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL OVERVIEW

Evidence from studies of animals and human adults suggests that the size of the eyeblink component of the startle reflex is systematically modulated by the organism's affective state. It has been shown that relative to a baseline response, the startle reflex is potentiated in the presence of a conditioned fear signal, as well as during the processing of unpleasant foreground stimuli. In contrast, the size of the eyeblink startle response is inhibited when pleasant foreground stimuli are being processed. This phenomenon (i.e., the emotional modulation of the startle reflex) has been interpreted in terms of the mediating effects of motivational systems in the brain: An unpleasant foreground stimulus activates the defensive motivational system, which results in a match between the defensive startle reflex and the organism's ongoing affective state, which in turn potentiates the startle response. Conversely, a pleasant foreground stimulus activates the appetitive motivational system, and thus a reflex-affect mismatch takes place and prompts an inhibited startle reaction.

The emotional modulation of the startle reflex has been reliably replicated in adults. In many of these studies, the emotion-modulated startle paradigm involves the presentation of pleasant, unpleasant, and affectively neutral pictures, during which an acoustic startle probe is presented. Studies examining the emotional modulation of startle in children and infants are scarce. The main purpose of the current research project was therefore to examine whether infants demonstrate a parallel of the adults' emotion-modulated startle effect. To examine the emotional modulation of startle in infancy, two modified versions of the adult emotion-modulated startle paradigm were developed and tested in two independent groups of 9-month-old infants. In Study 1, infants' startle responses to acoustic probes during the presentation of affectively neutral stimuli were compared to their startle responses during the presentation of pleasant and unpleasant stimuli. The pleasant, neutral, and unpleasant stimuli that were used were photographs of adult women appearing with happy, neutral, and angry facial expressions respectively. In Study 2, the characteristics of the modulated startle response were examined when infants were engaged in affectively pleasant, neutral, and unpleasant activities. The activities involved a game of peek-a-boo, a presentation of a spinning bingo wheel, and an arm restraint episode, respectively. In both studies, autonomic and behavioral data were also gathered in order to rigorously evaluate the contribution of emotion and other factors, such as attention, to the modulation of the startle reflex.

Another purpose of the current research project was to thoroughly examine the specific characteristics of the startle reflex in infants. These characteristics include the intensity of, and the latency to the peak response, and the frequency of its occurrence in response to the acoustic probes. In addition, the present research investigated the degree of habituation associated with the repetitive presentation of acoustic startle probes. Since no other study has systematically explored the characteristics of infants' startle responses in an emotion-modulated startle paradigm, the contribution of this research project is essential. It is believed that if emotional modulation of startle is present in infancy, startle paradigms can be used as an objective and reliable window into the emotional world of infants. Such paradigms could provide evidence that extends or converges with other behavioral studies of infant emotional responding. Indeed, the methods involved in eliciting the startle response are relatively unobtrusive and place only few demands on the participant, rendering startle paradigms ideal for research on the emergence of emotional responsiveness to various affective signals in infants. Furthermore, once established, these paradigms might be useful for the study of individual differences in infants' emotional behavior. This future line of research may have important implications for the understanding of normal and abnormal emotional development in infancy and early childhood.

LITERATURE REVIEW

The startle reflex

The startle reflex is a cross-species brainstem-mediated response that consists of a series of involuntary muscle movements occurring in reaction to an abrupt sensory event, such as an electric shock or a sudden burst of white noise (Landis & Hunt, 1939). Bodily reactions associated with the startle response include forward head and trunk movements, flexion of fingers, widening of the mouth, and contraction of the abdomen (Davis, 1984). While the whole-body startle reaction is typically recorded in animal studies, the most commonly used measure of startle in human research is the eyeblink response (Dawson, Schell, & Böhmelt, 1999). According to Landis and Hunt (1939), the eyeblink is considered the startle reaction that is the fastest, most reliable, and easiest to quantify. Indeed, rapid eye closure is one of the first components of the behavioral cascade that constitutes the startle reflex. Occurring 30 to 40 ms after stimulus onset in adults, the eyeblink startle response reflects an abrupt increase in tension in the *orbicularis oculi* muscle – the facial muscle that surrounds the eye. Given the short latency of the eyeblink startle reflex, it does not reflect the consequences of attention-switching or any extensive processing of the eliciting stimulus (Anthony & Graham, 1985). Rather, this brainstem response is thought to be a primitive defensive reflex that serves a protective, defensive function: Avoiding organ injury and orienting the organism toward dealing with possible threat (Lang, 1995).

The eyeblink startle response is often elicited by a short (e.g., 50 ms) and intense (95-110 dB) white noise with a fast rise time, although it is also possible to evoke the startle reflex using visual or tactile stimuli. The intensity and latency of the startle reflex can be noninvasively measured by monitoring the electromyographic (EMG) activity of the *orbicularis oculi* muscle, using miniature bioelectrodes placed just beneath the lower eyelid. The EMG waveform is then rectified and smoothed and scored for reflex size and latency. In addition, the probability of eliciting a startle response and the habituation of the response with repeated probes may be calculated.

It is now believed that the primary neural circuit underlying the eyeblink startle response to an abrupt noise involves a relatively small number of synapses (Davis, Walker, & Lee, 1999). Auditory nerve fibers synapse onto cochlear root neurons embedded in the auditory nerve. Axons from these cells project through the ventral acoustic stria and send projections to the nucleus reticularis pontis caudalis (PnC). Projections of cells in the PnC form the reticulospinal tract, which makes monosynaptic and polysynaptic connections with the spinal cord. These cells also project to the facial motor nucleus, specifically to areas that are critical for the eyeblink component of startle in humans. In animal studies, lesions of cochlear root neurons, the ventral acoustic stria, the PnC, or the reticulospinal tract eliminate the acoustic startle response. Research conducted by Davis and his colleagues has led to the hypothesis that the neurotransmitter mediating the startle response along the acoustic startle pathway is the excitatory amino acid glutamate (for review see Davis et al., 1999).

Startle reflex modulation: Terminology and paradigms

Over the last two decades, startle reflex paradigms have been increasingly used to study normal and abnormal emotional and attentional processes in humans. This growing interest can be attributed to numerous advantages of the startle reflex as a psychophysiological measure of inner processes and states. Perhaps most important is the idea that the startle reflex represents a physiological response that can be clearly manipulated. This plasticity of the eyeblink reflex provides researchers with the opportunity to utilize well-controlled paradigms to study various sensory, attentional, and emotional processes and their underlying neurobiological mechanisms. In addition, being mediated by a relatively simple neural circuit, the startle reflex has a short latency – a potentially important factor for understanding the mechanisms contributing to behavioral responses. The reflexive nature of the startle reflex also renders it invulnerable to intentional control and response biases. Finally, this bodily reaction is present at birth and does not require any voluntary motor performance or any other special requirements from participants, a consideration that is often relevant to the study of humans, and of particular significance in developmental research with infants. These factors are major incentives for utilizing startle modulation paradigms as objective and reliable tools in psychophysiological research.

A typical startle modulation paradigm includes the following parameters: A non-startling stimulus (“foreground stimulus”), a startle-eliciting stimulus (“startle probe”), and a time interval between the onset of the two stimuli (“interstimulus interval”). Numerous studies have demonstrated with humans and nonhuman animals that the duration of the interval separating the onset of the foreground stimulus and the startle probe is crucial in determining the direction of the startle modulation effect. Short interstimulus intervals (i.e., a few hundred milliseconds) result in startle inhibition (see Blumenthal, 1999 for review). This phenomenon is often called “prepulse inhibition”

(PPI), and it is considered as an operational measure of sensorimotor gating, reflecting an ability to reduce the impact of sensory stimuli (Braff & Geyer, 1990). Whereas prepulses or foreground stimuli presented at short intervals before the onset of the startle-eliciting stimulus have inhibitory effects, long interstimulus intervals (i.e., generally 800 ms or more) produce startle potentiation (see Putnam & Vanman, 1999 for review). This effect is thought to occur due to an orienting-attentional process (Graham, 1975). The two different types of startle modulation (i.e., inhibition and facilitation) may therefore signify the involvement of two distinct neurophysiological systems associated with the startle reflex. Yet, subsequent research has shown additional influences on startle modulation, such as selective attention (e.g., Anthony & Graham, 1985) and affective valence (e.g., Vrana, Spence, & Lang, 1988), the effects of which can be facilitatory or inhibitory depending on the specific characteristics (e.g., modality and valence, respectively) of the foreground stimulus to which attention is directed. The present research program focused on the modulation (facilitation and inhibition) of the startle reflex when emotion-eliciting stimuli and situations serve as foregrounds and startle probes are introduced after relatively long interstimulus intervals. This paradigm is referred to as "the emotional modulation of the startle reflex". However, selective attention effects that might influence startle modulation were also considered.

The emotional modulation of startle

Startle modulation has been a topic of inquiry since the beginning of the 20th century (e.g., Hilgard, 1933; Yerkes, 1905). However, the study of the emotional modulation of this reflex did not emerge until several decades later, alongside the growing interest in understanding classical conditioning. In a classic study, Brown, Kalish, and Farber (1951) conditioned a group of rats to associate a light and a buzzer with an imminent electric shock. The affective state of the rat after exposure to the conditioning stimulus (light or tone) has been termed “fear”. The intensity of the startle reflex elicited by a startle probe (shots from a toy pistol) in the conditioned group of rats was compared with the startle response of control rats that had not been conditioned. Results indicated that the startle response in the experimental group was significantly larger than in the control group. These data suggest that presenting the startle probe in the presence of a conditioned fear state, namely a cue that has previously been paired with a shock, can lead to augmentation of the acoustic startle reflex. Since this initial work, fear-potentiated startle has been demonstrated in many experimental paradigms with animals (e.g., Davis & Astrachan, 1978). More recently, fear-potentiated startle has also been demonstrated in humans, using procedures that employ the anticipation of aversive events such as electric shocks, or air puffs directed at the larynx (e.g., Grillon, Ameli, Woods, Merikangas, & Davis, 1991, 1993; Grillon et al., 1999; Hamm, Greenwald, Bradley, & Lang, 1993; Lipp, Sheridan, & Siddle, 1994).

Fear-potentiated startle is mediated by the central nucleus of the amygdala. The amygdala, located within the temporal lobes of the brain, is known as a critical structure in the mediation of a variety of emotional behaviors (e.g., Aggleton & Mishkin, 1986; Everitt & Robbins, 1992; Ursin, Jellestad, & Cabrera, 1981). Specifically, there is accumulating evidence to suggest that the amygdala is highly relevant for the expression of negative affect and aversion-driven behaviors (Cahill & McGaugh, 1990). From the central nucleus of the amygdala there is a direct projection to the PnC (Rosen, Hitchcock, Sananes, Miserendino, & Davis, 1991). As described earlier, projections of cells in the PnC form the reticulospinal tract, which makes synapses in the spinal cord. These cells also project to areas in the facial motor nucleus that are critical for the eyeblink startle response. Lesions of the amygdala (Campeau & Davis, 1995; Hitchcock & Davis, 1986, 1987), as well as lesions along the amygdala-PnC pathway (Hitchcock & Davis, 1991) block the expression of fear-potentiated startle. These findings are in line with LeDoux's work showing that the lateral nucleus of the amygdala receives fear-conditioning stimulus information (LeDoux, Cicchetti, Xagoraris, & Romanski, 1990), which may be then relayed to the central nucleus either directly or through the basal and accessory basal nuclei (Pitkänen et al., 1995). While most of the research considering the role of the amygdala in startle modulation has involved animal studies, the amygdala has also been implicated in fear conditioning and fear-potentiated startle in humans. Patients with lesions of the amygdala have been reported to have deficits in classical fear conditioning (Bechara et al., 1995; LeBar, LeDoux, Spencer, & Phelps, 1995). Patients with unilateral left, but not right, temporal lobectomy including the amygdala failed to show fear-potentiated startle response (Funayama, Grillon, Davis, & Phelps, 2001). Left lateralization of amygdala activation during threat was also found in a functional Magnetic Resonance Imaging (fMRI) study with healthy participants (Phelps et al., 2001).

Studies of fear-potentiated startle prompted the notion that the emotional valence of a foreground context can modulate the startle reflex. This idea was tested and confirmed by Vrana et al., (1988) and has been repeatedly replicated in several independent laboratories (Bradley, Codispoti, Cuthbert, & Lang, 2001; Bradley, Cuthbert, & Lang, 1990, 1993; Bradley & Lang, 2000; Bradley, Lang, & Cuthbert, 1993; Dichter, Tomarken, & Baucom, 2002; Ehrlichman, Brown, Zhu, & Warrenburg, 1995; Jansen & Frijda, 1994; Kaviani, Gray, Checkley, Kumari, & Wilson, 1999; Kaviani, Wilson, & Checkley, 1998; Miltner, Matjak, Braun, Diekmann, & Brody, 1994; Sabatinelli, Bradley, & Lang, 2001). Perhaps the most extensively used emotion-modulated startle paradigm involves the presentation of pleasant, unpleasant, and affectively neutral pictures, during which an acoustic startle probe is presented in the form of a 50 ms burst of white noise with instantaneous rise time and a sound pressure level (SPL) of between 95 to 110 dB. In many of the studies employing this paradigm, the pictures have been taken from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 1997a). The IAPS includes photographs of people, animals, objects, events, and scenes that have been rated on the dimensions of affective valence and arousal (Greenwald, Cook, & Lang, 1989; Lang, Greenwald, Bradley, & Hamm, 1993). According to Lang (1995), these two dimensions organize the entire emotional world of humans.

Using this paradigm, a replicable finding has been that the size of the startle blink response is systematically and differentially modulated according to the affective valence of the foreground picture (Bradley et al., 1990, 1993, 2001; Cuthbert, Bradley, & Lang, 1996; Dichter et al., 2002; Sabatinelli et al., 2001; Vrana et al., 1988). Relative to the size of the startle response when viewing affectively neutral pictures (e.g., household objects), acoustic startle probes presented during unpleasant pictures (e.g., pictures of attack and mutilation) evoke a potentiated startle blink, whereas identical probes presented during the presentation of pleasant pictures (e.g., pictures depicting erotica and beautiful nature scenes) elicit an inhibited reflex reaction. Emotional modulation of the startle reflex has been observed not only with acoustic startle probes (e.g., Vrana et al., 1988), but also with probes that were visual (Bradley et al., 1990), or tactile (Hawk & Cook, 1997).

Above and beyond the modulation of the startle reflex as a function of the valence of the emotional context in which the startle-evoking probe occurs, the intensity of the startle response varies according to the level of arousal exhibited by the individual. In general, the reciprocal effects for pleasant and unpleasant stimuli on reflex modulation become more pronounced in the context of more arousing stimuli. Reflex modulation – potentiation for unpleasant stimuli and inhibition for pleasant stimuli – was found to be greatest for highly arousing stimuli (Cuthbert et al., 1996). In adults, there is evidence showing that the most arousing unpleasant pictures are those involving direct danger and threat, whereas the most arousing pleasant pictures are the ones depicting erotic scenes (Lang, Bradley, Drobes, & Cuthbert, 1995). In addition, the arousal dimension was found to have an impact on the level of the *orbicularis oculi* muscle activity prior to blink onset. In adults, baseline EMG activity was found to be higher during arousing events, such as the processing of affectively positive and negative stimuli, than during non-arousing events, such as the processing of affectively neutral stimuli (Bradley et al., 1990; Cook, Davis, Hawk, Spence, & Gautier, 1992).

Research has demonstrated that during the emotion-modulated startle paradigm, the activity of a number of physiological systems covaries significantly with the two dimensions of affective valence and arousal (Bradley et al., 2001; Greenwald et al., 1989; Lang et al., 1993; Winton, Putnam, & Krauss, 1984). For example, differences in affective valence were found to be associated with facial muscle activity. Thus, when valence ratings are ranked from the most to the least unpleasant, corrugator (“frown”) EMG activity decreases, and zygomatic (“smile”) EMG activity increases. Heart rate (HR) was also reported to be responsive to differences in affective valence: Unpleasant pictures generally prompt marked HR deceleration during viewing, whereas HR acceleration occurs during the viewing pleasant pictures. Rated arousal, on the other hand, was found to be positively associated with skin conductance, viewing time, and ratings of interest, regardless of the valence of the foreground stimulus. An arousal augmentation effect has also been noted in the P300 component of the cortical event-related potential, recorded in response to startle probes in the context of affective (pleasant and unpleasant) versus neutral pictures (Schupp, Cuthbert, Bradley, Lang, & Birbaumer, 1993).

Although the use of the picture-viewing paradigm, particularly the IAPS, to study the emotional modulation of the startle reflex has proven useful, significant startle modulation has also been obtained using other types of foreground stimuli. These include evocative video film clips (Jansen & Frijda, 1994; Kaviani et al., 1999), pleasant and unpleasant odors (Erlichman et al., 1995; Kaviani et al., 1998; Miltner et al., 1994), pleasant and unpleasant sounds (Bradley, Zack, & Lang, 1994), and emotional text (Spence & Lang, 1990). As long as the arousal level of the foreground stimuli was high, the startle reflex was potentiated when the affective valence of the stimuli was negative, compared with stimuli having positive valence.

According to Lang and colleagues, emotional modulation of the startle reflex does not require the actual presence of a perceptual stimulus, but instead it indicates the state affect associated with processing affective stimuli (Bradley, Cuthbert, & Lang, 1999). This conclusion was drawn from studies in which emotional modulation of startle was obtained even when startle probes were presented when the affective foreground stimuli were no longer displayed. When foreground stimuli were presented for relatively short time periods, startle probes elicited after their offset produced the typical pattern of emotional modulation (Codispoti, Bradley, & Lang, 1996; Globisch, Hamm, Esteves, & Öhman, 1994). For longer presentations of foreground stimuli, emotional modulation was demonstrated if participants were explicitly instructed to continue to imagine the affective foregrounds after their actual offset (Bradley et al., 1993; Schupp, Cuthbert, Bradley, Birbaumer, & Lang, 1997). Similarly, emotion-modulated startle effect was obtained when participants were asked to enhance or suppress negative emotion elicited by the IAPS (Jackson, Malmstadt, Larson, & Davidson, 2000).

The emotional modulation of startle does not appear to show habituation over time. Despite a marked general habituation of the startle reflex itself over repetitive presentation trials of the same startling stimuli (Hirano, Russell, Ornitz, & Liu, 1996), it has been shown that startle potentiation and inhibition persist, at least when the affective foreground stimuli are repeated in the same experimental session. Bradley and her colleagues examined whether picture repetition may cause a decrease in the degree to which the eyeblink response is potentiated when probes are presented during processing of unpleasant pictures or inhibited when viewing pleasant pictures (Bradley, Gianaros, & Lang, 1995; Bradley et al., 1993). They found no such habituation effect when the same foreground stimuli were presented during a single experimental session, nor when the repetition occurred over two sessions that were one week apart. In both experiments, a significant emotional modulation of the startle reflex was found regardless of whether the foreground pictures were presented for the first or second time. Data from Davidson's laboratory have shown similar results with respect to the temporal stability of the emotion-modulated startle effects when a repetition occurred in the same assessment (Sutton, Davidson, Donzella, Irwin, & Dottl, 1997). Nonetheless, when using the same foreground stimuli to manipulate affective state at two assessments separated by a few weeks, data from Davidson's and other laboratories have failed to replicate Bradley's findings by showing poor stability of the emotion-modulated startle response (Larson, Ruffalo, Nietert, & Davidson, 2000; Manber, Allen, Burton, & Kaszniak, 2000).

A motivational account for the emotion-modulated startle

Lang and his colleagues have proposed a motivational priming mechanism to account for the emotional modulation of the startle reflex (for reviews see Bradley et al., 1999; Lang, 1995; Lang, Bradley, & Cuthbert, 1990, 1992, 1997b, 1998). The motivational account is rooted in a number of earlier theories (Schneirla, 1959; Konorski, 1967; Dickinson & Dearing, 1979) that view emotions as products of Darwinian evolution. Although the emotional world of humans is rich and complex, it is postulated that the evolutionary foundation of emotion has a simpler, fundamental, two-factor motivational organization aimed at promoting physical survival. In this view, all emotions are mediated or driven by two functionally opposing brain systems: The appetitive system and the defensive system. Each system adaptively responds to appetitive or aversive stimulation respectively, determining an evolutionary-adaptive course of action. Thus, when organisms respond to appetitive stimuli or are engaged in pleasant activities that might promote physical survival, the appetitive brain system is activated, which is prototypically expressed by behavioral approach. Conversely, the defensive motivational system is activated when organisms respond to aversive stimuli or are engaged in unpleasant activities that might reflect the proximity of threat. The activation of the defensive system is prototypically expressed by behavioral withdrawal, avoidance, or escape.

Investigators now possess considerable knowledge about the neural structures and pathways involved in defensive reactivity. The neural network of the defensive motive system is indeed similar to the neural circuit depicted earlier in the context of the fear-potentiated startle reflex. This system can be traced starting from the stages of sensory input, proceeding through the key structure of the amygdala, and on to the autonomic and motor effectors. The unique role of the amygdala in processing aversive stimuli has been demonstrated in a recent positron emission tomography (PET) study that revealed amygdala activation in response to negative pictures, but not to positive pictures, relative to affectively neutral pictures (Paradiso et al., 1999). The literature regarding the neurophysiological circuit that defines the appetitive motive system is relatively sparse and is confined to animal research. However, there is evidence to suggest that startle inhibition by appetitive cues might not be mediated by the amygdala, but by a different neural substrate. Research with rats demonstrated a reduction in startle during appetitive conditioning (Schmid, Koch, & Schnitzler, 1995) that was blocked by lesions of the nucleus accumbens, but not by lesions of the amygdala (Koch, Schmid, & Schnitzler, 1996). In contrast to the dimension of valence, the dimension of arousal is not represented by a separate brain system, but rather levels of arousal reflect variations in the activation of each motivational system. Thus, arousal does not have an independent, unitary effect on behavior: Modulation of the defensive reflex increases with arousal, but the direction of the effect is different, depending on the motivational system (appetitive or defensive) that is engaged.

As well as organizing the physiological and behavioral responses to specific affect-eliciting input, the active motivational system - appetitive or defensive - also exerts a modulatory effect on other processing operations in the brain. In general, memory associations, representations, and action programs that are linked to the engaged motivational system, are specially “primed”, resulting in a higher probability of access as well as potentially greater output strength than other information. Conversely, mental events and programs linked to the non-engaged system have a reduced probability and strength of activation.

The startle response is seen as a protective-defensive reflex, which presumably activates the defensive motivational system. Consistent with the motivational priming model, the modulation of this reflex is dependent upon the match between the organism’s ongoing state and the defensive motivational system activated by the startle-inducing stimulus. Thus, evoking the defensive startle reflex in the context of an ongoing defensive motivational state (i.e., when the organism is in a fear state or is reacting to an unpleasant foreground stimulus) results in a reflex-affect match, which in turn prompts a reflex response that occurs considerably faster and is of significantly greater intensity. In contrast, the startle reflex is slower and reduced in size when the organism is processing an appetitive stimulus (i.e., is in states of pleasure), since a mismatch has occurred between the ongoing positive affect of the individual and the defensive nature of the startle reflex. Importantly, both priming effects – potentiation and inhibition of responding – are expected to be enhanced with increasing levels of arousal exhibited by the organism.

As described earlier, the motivational priming account for the emotional modulation of startle has gained wide support from studies utilizing the picture-viewing paradigm. In addition, the motivational explanation has been encouraged by research in which the startle probe was administered unilaterally. Such laterality research has repeatedly demonstrated that each brain hemisphere is associated with a different motivational system. Specifically, it has been shown that the right hemisphere of the brain is associated with the processing and expression of withdrawal-related emotions and behaviors, whereas the left hemisphere is specialized for the processing and expression of approach-related emotions and behaviors (Davidson & Fox, 1988). Given the way in which affective processing is lateralized in the brain, if the motivational account to the startle reflex is correct, the largest enhancement in startle response should be obtained not only when individuals are engaged with aversive stimuli (as in the typical emotion-modulated paradigm), but also when the startle probe is processed by the right hemisphere. On the other hand, processing of the startle probe by the left hemisphere should reduce the intensity of the startle response. These hypotheses have been confirmed in a number of studies. When startle probes were presented to the left ear (i.e., the probes were processed predominantly by the right hemisphere), the affective modulation of the startle reflex took the expected form of larger blink reflexes in the context of aversive, compared with pleasant stimuli (Bradley, Cuthbert, & Lang, 1991, 1996). In contrast, when probes were presented to the right ear, no significant effects of emotional context have been found. Similar findings were obtained when unilateral tactile (air puff), rather than acoustic, startle probes have been presented (Hawk & Cook, 1997).

The modulated startle response occurs shortly after probe presentation (30-40 ms in adults, depending on attributes of the probe and foreground stimuli), implying that unlike many other emotion-related responses (e.g., facial expressions), the emotional modulation of startle has no conscious or intentional mechanism, and is not part of social-affective communication (Bradley et al., 1999). Nevertheless, from an emotional priming perspective, this paradigm is invaluable to the study of human emotion in that it permits an objective measure of the foreground task's effect on the organism's emotional state. Specifically, potentiation of the eyeblink startle response may indicate the ongoing activation of an aversive motivational system, and inhibition of the startle reaction may signal that the foreground task activated the appetitive motivational system. The notion that startle responses can track the organism's valence dimension of emotion or motivational state is particularly attractive in psychophysiological research, given the lack of such a property among traditional psychophysiological indices, such as electrodermal, cardiovascular and facial electromyographic measures (Grillon & Baas, 2003). Although it is possible to probe individuals' emotional state using self-report measures, verbal reports are often vulnerable to individual perception, demand characteristics, and intentional distortions. Moreover, self-report measures cannot be used in nonverbal populations, such as young infants. Given that the elicitation of the startle reflex does not require verbal abilities or voluntary motor control, startle paradigms can also potentially be used with infants. If the neural systems mediating emotional modulation of startle are functional in infancy, the startle paradigm might be a promising technique for probing infants' emotional state and responsivity to affective signals. In this sense, such paradigms could provide evidence that extends or converges with other behavioral studies of infant emotional responding.

Emotional modulation of startle in infants

The blink component of the startle reflex is present at birth, although infants have longer blink latencies than adults and may require probe stimuli of slightly longer durations to elicit the reflex (Anthony, Zeigler, & Graham, 1987). The startle reflex is non-invasive, not overly difficult to measure, and in addition has the advantage of imposing few requirements from participants. To date, the method of using the eyeblink startle reflex to probe ongoing stimulus processing has been used successfully in a small number of studies with young infants in which sensory and modality-selective attentional influences have been examined (e.g., Anthony & Graham, 1983; Balaban, Anthony, & Graham, 1985; Richards, 1993, 2000).

The investigation of the startle response in infants during emotion-eliciting foregrounds has been particularly scant, perhaps due to the fact that the foreground stimuli that have been typically used when studying the emotional modulation of startle in adults (i.e., the IAPS) are often meaningless and inappropriate for young infants. The adaptation of the emotion-modulated methodology for startle research in infancy requires the crucial consideration of what types of stimuli will be emotionally salient to infants. This requires the understanding of the development of positive and negative affect, and how affective states can be elicited throughout infants' emotional development.

The development of positive affect in the first year of life typically follows a general trajectory that can be described as follows. In the early months of life, smiling can be elicited by social interaction, such as human voice or a moving human face (Emde & Harmon, 1972). During the third month of life, expressions of joy are often clearly visible when infants are engaging in social play with their parents (Izard et al., 1995). By 3-4 months of age, more arousing and intense stimulation (especially social stimulation) may elicit laughter (Field, 1982). In the latter months of the first year, positive emotions can be elicited during interactions which are more complex and structured than those in the first months of life. For example, a common feature of playful parent-child interactions in older infants is the “peek-a-boo” game. The success of the game in eliciting the appropriate positive affect depends on the infant’s ability to integrate a variety of information. In order to create the set of expectancies on which the game depends, the infant requires some degree of object permanence and an awareness of spatial and temporal structuring (Bruner & Sherwood, 1976). These abilities are part of a set of abilities that are maturing during the latter months of the first year of life. Indeed, many studies have supported the notion of a major transition in infant development over the time period around 8-12 months of age (for review, see Kopp & Neufeld, 2003). This literature points to the emergence of “active control processes” related to joint attention, controlled gestures, and mastery behaviors (Kopp & Neufeld, 2003). Each of these capacities has been seen to undergo rapid development in the last third of the first year, and may provide the basis for later developing emotion regulatory capacities.

In terms of the development of negative affect, Lewis (2000) views distress as a primary emotion that is present from birth. From this perspective, by around 6 months of age, this initial expression of generalized distress has differentiated into sadness, disgust, fear, and anger. Over the latter half of the first year, there continue to be important changes in both the expression and regulation of negative affect. While the psychological transitions of the latter months of the first year of life were noted above as having important implications for the expression of positive affect, these transitions also have significant impact on the expression of negative affect (Kopp & Neufeld, 2003). For example, the emergence of controlled actions in response to unfamiliarity and the increasing selectivity of affiliation with specific individuals are associated with changes in the phenotypic expression of fear of novelty in this age range (Bronson, 1972; Sroufe, Waters, & Matas, 1974). During these months there is also an increase in the use of strategies for regulation of negative affect in response to frustration (Kopp, 1989; Stifter & Braungart, 1995).

As outlined above, there are important changes in emotion expression occurring in the last few months of the first year. The use of psychophysiological measures to probe emotional state may be particularly important over this time period as infants begin to develop rudimentary strategies for regulating both positive and negative affect. However, there is very little work examining the physiological processes associated with the expression and experience of emotion in the second half of the first year of life. The current study is an attempt to add to that literature by employing the emotion-modulated startle paradigm to probe the emotional experience of 9-month-old infants. Given that this age point falls in a period of rapid development of the emotional world of infants, the examination of physiological processes associated with emotion-eliciting stimuli at 9 months of age may aid the understanding of the developmental processes that are occurring over the latter half of the first year of life.

To date, the examination of the emotional modulation of the startle reflex in infancy has been particularly scant. Balaban (1995) studied emotion-modulated startle in 5-month-old infants using static visual presentation of happy and angry facial expressions as pleasant and unpleasant stimuli respectively. Balaban (1995) found that 5-month-old infants demonstrate a pattern of startle modulation that is similar to the pattern of emotion-modulated startle in adults. Specifically, she reported that the magnitude of 5-month-old infants' startle blink responses to acoustic probes were larger when the infants were viewing pictures of angry faces than when viewing happy faces. There was a non-significant trend for a difference in response latency as a function of affect condition, with faster blinks to probes presented during angry pictures and slower blinks to probes presented during happy pictures.

Balaban's (1995) study represents a first attempt to adapt the emotion-modulated paradigm to explore whether affective processes like those in adults are functional early in development. According to Balaban (1995), her study indeed demonstrated not only that the emotional modulation of startle is present and functional early in development, but also that responses to emotional signals are organized at an early stage around appetitive and defensive systems. A similar idea was also proposed by Davidson and Fox (1988), who suggested that connections between limbic and frontal cortical areas, which are partly functional at birth, mediate an underlying organization of approach versus withdrawal tendencies.

Nonetheless, it is important to note that it is somewhat questionable whether the pleasant and unpleasant stimuli that were used in Balaban's (1995) study were indeed appropriate for 5-month-old infants. Several researchers have suggested that emotionally appropriate behavioral responses are necessary in order to infer that infants detect affective information (e.g., Fernald, 1993; Nelson, 1987). Based on this criterion of appropriate, differential responses, researchers have shown that 3-month-old infants can detect affective information when conveyed dynamically through maternal facial and vocal expressions (Haviland & Lelwica, 1987; Tronick, Ricks, & Cohn, 1982). Fernald (1993) reported differences in 5-month-olds' facial affect to approving and disapproving speech. Other research has suggested that the ability to discriminate and categorize emotional expressions may develop several months later. For example, it has been demonstrated in several different laboratories that the ability to discriminate among a variety of facial expressions depicted in both photographs and videotapes is developed by 7 months of age (Kestenbaum & Nelson, 1990; Ludemann & Nelson, 1988; Soken & Pick, 1999). Thus, it is possible that the infants who participated in Balaban's (1995) study were too young to detect the valence of the affective slides that were presented to them as foreground stimuli. Demonstration of differential responses to happy and angry expressions – even if in accord with the emotion-modulated paradigm - does not provide sufficient evidence that infants recognized or were responding to the emotional content of the stimuli. Indeed, Balaban (1995) reported no significant differences in her participants' facial expressions during the viewing of the happy and angry faces. Similarly, she reported no differences in pre-blink baseline EMG activity during the viewing of happy or angry faces, relative to neutral faces. This is not consistent with adult studies, in which stimuli with emotional content typically evoke increased baseline *orbicularis oculi* activity, a pattern ascribed to increased arousal (Bradley et al., 1990; Cook et al., 1992). Taken together, these observations suggest that in Balaban's (1995) study the differential modulation of the startle reflex in response to happy and angry facial expressions was not a result of the specific affective information, but may be due to cognitive processing of specific facial features (Caron, Caron, & Myers, 1985; Kestenbaum & Nelson, 1990; Ludemann, 1991; Serrano, Iglesias, & Loeches, 1995) or variance in the degree of familiarity of the expression (Kagan, 1974). Ornitz (1999), in commenting on Balaban's (1995) findings, suggested that at 5 months of age, infants have less experience with angry than neutral or happy expressions. Indeed, studies have shown that by 7 months of age, infants have typically been exposed to happy expressions more frequently than angry ones (Malatesta, Grigoryev, Lamb, Albin, & Culver, 1986; Malatesta & Haviland, 1982). It is possible that infants in Balaban's (1995) study directed unequal attention to the different stimulus types due to differential levels of experience with the facial expressions presented. This would suggest that familiarity or unfamiliarity, rather than the actual emotional content of the face stimuli, was primarily responsible for the modulation of the startle reflex.

Schmidt and Fox (1998a) studied the emotional modulation of startle in a group of 9-month-old infants. Their paradigm included the presentation of an acoustic startle probe while infants and their mothers were alone in the experimental room (a baseline condition), as well as during a stranger approach (fear-potentiated condition). In this study, differences in the intensity of the startle response between baseline and fear-potentiated conditions were not reported for the entire group of participants. Rather, the emotion-modulated effect was analyzed separately for three groups of infants with different temperamental characteristics. Infants in one group had displayed high motor activity and negative affect at 4 months of age, whereas in the second group, infants had displayed high motor activity and positive affect. The third group consisted of infants who displayed low levels of motor activity and affect at 4 months of age. Schmidt and Fox (1998a) reported that the high motor/high negative group of infants exhibited a significantly greater fear-potentiated startle responses at 9 months of age compared with the high motor/high positive group, even though there were no differences among the groups in baseline startle responses. These findings were taken to support the contention that a low threshold for arousal in the amygdala (particularly the central nucleus) is one of the physiological markers of a high motor/high negative temperament. Furthermore, since these temperamental characteristics have been found to predict behavioral inhibition in early childhood (Calkins, Fox, & Marshall, 1996; Kagan & Snidman, 1991), it has also been suggested that a hypersensitive amygdala may make the high motor/high negative infants more vulnerable to the development of behavioral inhibition (Schmidt & Fox, 1998a).

The study of Schmidt and Fox (1998a) made a significant contribution to the existing knowledge about emotional modulation of startle in infancy. Moreover, this study represents an intriguing attempt to relate the degree of activation of the defensive motivational systems to individual differences in temperamental reactivity. Yet, as in Balaban’s (1995) study, a number of methodological and conceptual issues in Schmidt and Fox’s (1998a) study call for further research to be conducted in a more rigorous and extended fashion. First, unlike the emotion-modulated startle paradigm developed by

Lang and his colleagues (Bradley et al., 1990; Greenwald, Bradley, Cuthbert, & Lang, 1990; Vrana et al., 1988), Schmidt and Fox (1998a) did not use any appetitive foreground, but only an aversive situation, which was compared to a baseline epoch. An interesting addition would have been to examine infants' startle responses during engagement with a pleasant situation, and to assess whether the patterns of this response vary between infants as a function of temperament. Second, the researchers did not include behavioral or other physiological measures (e.g., heart rate) in their study to assist in the determination of whether the desired affect (i.e., fear) was indeed induced during the stranger approach. Third, the findings obtained in this study were reported for highly selected temperament groups, rendering the general understanding of infants' startle responses in a fear-potentiated paradigm somewhat limited. Furthermore, due to a relatively high rate of startle data loss, the final sample size of this study was small. And finally, in order to measure the eyeblink response to the acoustic probe, the authors attached one electrode to the supraorbital area next to the right eye, and attached another electrode to the outer canthus of the same eye. Although this unconventional placement was shown to be viable for measuring emotion-modulated startle responses in adults (Schmidt & Fox, 1998b), it is very rarely used and it is therefore somewhat difficult to interpret results from this placement in the context of the wider startle literature. Thus, while Schmidt and Fox (1998a) was a groundbreaking attempt to examine emotion-modulated infant startle responses, additional research on the emotional modulation of startle in infants is clearly still required and potentially valuable.

A small number of studies of emotional modulation of startle have been conducted with school-age children. McManis, Bradley, Cuthbert, and Lang (1995) studied 7- to 10-year-old children in the picture-viewing paradigm and were unable to demonstrate the adult pattern of modulation. However, when responses were segregated by gender, girls showed the expected (adult-like) potentiation of startle while viewing unpleasant pictures, whereas boys actually showed inhibition of startle in this condition. Cook, Hawk, Hawk, and Hummer (1995) also were unable to demonstrate the adult pattern of startle modulation to affectively valenced script-induced imagery in school-age children. Virtually identical startle magnitude was found during imagery designed to evoke feelings of pleasure, joy, sadness, fear, and anger. Further, the startle responses of children who scored higher on a fear survey were smaller during unpleasant compared with pleasant imagery. This significant affective valence by fear interaction was opposite to that found in adults (Cook, Hawk, Davis, & Stevenson, 1991). It is possible that in children, particularly boys (McManis et al., 1995) and more fear-prone children (Cook et al., 1995), greater attention to unpleasant pictures or imagery draws attentional resources away from the startling stimuli, resulting in smaller startle responses (Ornitz, 1999). Further investigation is clearly required to understand the effects of emotional signals on startle modulation and their interaction with attention during infancy and childhood.

Startle modulation by attention

Graham and her associates have put forth the idea that the startle reflex is modulated according to the amount of attentional resources allocated to a primary, foreground task. In a series of experiments employing startle probe methodology, Graham manipulated stimuli, tasks, and instructions given to participants in order to guide their attentional focus (e.g., Anthony & Graham, 1983, 1985; Bohlin & Graham, 1977; Hackley & Graham, 1984; Silverstein, Graham, & Bohlin, 1981). Data from these studies have highlighted several important phenomena concerning the association between attention and startle blink modulation. For example, instructions to attend to the startle probe itself prompt a potentiated startle response (e.g., Bohlin & Graham, 1977; Hackley & Graham, 1984; Silverstein et al., 1981). In contrast, as the nature of the primary task requires an increasing amount of attention, startle responses are inhibited, presumably due to a decrease in the amount of attentional resources directed to the startle probe.

Another series of experimental studies examined the modulation of the startle reflex as a function of the match in sensory modality between the foreground task and the startle probe (e.g., Anthony & Graham, 1983, 1985; Hackley & Graham, 1984). In these studies, participants attended to either a visual or auditory foreground task while startle probes were administered in either the same or the alternate sensory channel. Anthony and Graham (1985) reported that both infants and adults demonstrate a slower and smaller startle response when there is a mismatch between the probe modality and the modality of the foreground stimulus (e.g., an acoustic probe with a pictorial slide foreground). This inhibition of response was interpreted in terms of limited attentional resources that are allocated *a priori* according to modality. Thus, when participants engage in a task requiring modality-specific attention, fewer resources are available to the alternate modality through which the probe stimulus is presented, producing an inhibited startle response.

It is important to note that the selective attention theory for the modulation of the startle reflex has been challenged by emotion-modulated startle studies that manipulated the modality in which the startle probe was presented. To date, emotional modulation in the picture-perception paradigm has been investigated using acoustic (e.g., Vrana et al., 1988), visual (Bradley et al., 1990; Erickson, Levenston, Curtin, Goff, & Patrick, 1995), and tactile (Hawk & Cook, 1997) startle probes. In line with the motivational priming account and in contrast to the selective attention theory, a consistent finding derived from these studies is that regardless of probe modality, the startle reflex is augmented in the context of unpleasant picture viewing, and inhibited when viewing pleasant pictures. Thus, it has been demonstrated that the selective attention hypothesis cannot explain the reflex modulation by affective valence. However, in experiments where emotional excitation is controlled or minimized, modality appears to determine a significant portion of the response variance. In effect, both parameters (modality and affect) may be natural categories into which the brain organizes information and according to which responses are deployed.

A further aspect of Graham's research program has concerned the interest value of the foreground stimulus. Anthony and Graham (1985) proposed that more interesting foreground stimuli engage attention to a greater extent than do less interesting foreground stimuli, and thus a greater cross-modality startle response inhibition is expected with interesting than with dull foregrounds. This hypothesis was confirmed by Simons and Zelson (1985), who found that participants displayed a significantly inhibited startle response to an acoustic probe when they viewed interesting photographic slides (i.e., a varying series of attractive nude men and women), compared with viewing a slide with dull content (i.e., a wicker basket) that was repeatedly presented. A similar pattern of results was obtained with infants in Anthony and Graham's (1983, 1985) studies. These investigators tested attentional allocation in 4-month-old infants during visual or auditory foreground stimuli. During the visual foregrounds (solid-color slides and slides of smiling faces), the infants' startle responses elicited by light flashes were potentiated and their startle responses elicited by sounds were inhibited. During auditory foregrounds (continuous tones or musical melodies), visually elicited blinks were inhibited and acoustically elicited blinks were potentiated. These effects were more pronounced during the more interesting foregrounds (faces and melodies).

This line of research was challenged by Lang and his colleagues (Lang et al., 1992), who stressed that the interesting stimuli used in Simons and Zelson's (1985) study are not motivationally neutral. Erotic pictures were reliably classified as both highly pleasant and arousing (Greenwald et al., 1989), therefore they are expected to prompt inhibition of startle responses due to the mismatch between the appetitive motivational system activated by the pleasant foregrounds and an aversive startle probe. In this sense, the salient foregrounds (e.g., smiling faces) used in Anthony and Graham's (1983) study are also not motivationally neutral. On a similar note, in the study conducted by Vrana et al. (1988), participants reported the pleasant and unpleasant slides to be equal in interest and significantly more interesting than the neutral pictures. Yet, regardless of interest level, and in contrast to Graham's attention theory, participants displayed a potentiated startle response during the presentation of unpleasant slides, and inhibited startle response during the presentation of pleasant slides.

The selective attention explanation for startle modulation has been further challenged by research in which individuals' heart rate (HR) was collected during the picture-viewing paradigm. According to Graham's selective attention theory, startle potentiation during the viewing of unpleasant pictures indicates an attenuated mismatch between the acoustic probe and the visual foreground stimulus, which can be the result of less attention allocation to unpleasant pictures. Startle inhibition during the viewing of pleasant pictures may indicate an enhanced probe-foreground modality mismatch, which is due to greater attention allocation to pleasant pictures. It is well established in the attention literature that in both infants and adults, the psychological state of orienting and attention to external stimulation is typically associated with HR deceleration, whereas stimulus rejection is typically associated with HR acceleration (Graham, Anthony, & Zeigler, 1983; Graham & Clifton, 1966; Lacey & Lacey, 1974; Porges, 1992). Thus, if Graham's hypothesis was correct, pleasant pictures would be associated with HR deceleration, and unpleasant pictures would be associated with HR acceleration. In contrast, various studies have shown that greater HR deceleration occurs during the presentation of unpleasant pictures (Bradley et al., 1990; Lang et al., 1990), whereas greater HR acceleration is associated with viewing pleasant pictures (Greenwald et al., 1989; Lang et al., 1993). These findings question the selective attention theory and add support to the motivational account for startle modulation by emotion.

In summary, there is evidence to support both the motivational account and the attention-based explanation of startle modulation. It may be that motivation and attention are part of the same system, such that the startle reflex response to an acoustic probe is inhibited when processing pleasant, interesting visual stimuli, and is potentiated in the context of unpleasant stimuli (Bradley et al., 1999). Alternatively, it is possible that two different mechanisms are involved in the inhibition of the startle caused by increased attention allocation compared with startle inhibition due to the activation of an ongoing appetitive motivational system (Lang, 1995). It is also possible that both modality and affect influence the startle response, in which case the modulation of the startle reflex is mainly dependent on the relative strength of the organism's emotional excitation.

STUDY 1

Overview

In this study, a modified version of the adult emotion-modulated startle paradigm was tested in a group of 9-month-old infants. This paradigm included delivery of an acoustic startle probe during the presentation of affectively pleasant, neutral, and unpleasant foreground stimuli. The stimuli used in this study were photographs of adult women appearing with happy, neutral, and angry facial expressions respectively.

The purpose of this study was to examine whether infants demonstrate a parallel of the emotion-modulated startle effect that has been well replicated in adults. As well, the specific characteristics of infants' startle response were systematically explored, including the intensity of and the latency to the peak response, the frequency of its occurrence in response to the acoustic probes, and the degree of habituation in the intensity of the startle response associated with the repetitive presentation of acoustic probes. Finally, the current study included the measurement of autonomic reactivity and looking time during the emotion-modulated startle paradigm in order to facilitate the understanding of the role of attention in infants' startle modulation.

Methods

Participants

Thirty-two 9-month-old infants (± 7 days) participated in this study. As outlined earlier, this age point was chosen to aid the understanding of the rapid and salient developments that occur in the emotional world of infants over the latter half of the first year of life. Three additional infants were seen at the laboratory, but neither EMG nor cardiac data could be obtained from these participants due to high levels of distress displayed by the infants.

The pool of participants was recruited through commercially available mailing lists. Families with young infants who live in the Washington, D.C. metropolitan area were mailed a cover letter (see Appendix A), a brief survey (see Appendix B), and a postage-paid business reply envelope. The survey requested information about the birth of their child, including method of delivery, birth complications, number of days in the hospital, and any illness or medical problems. In addition, information was requested on parent ethnicity. Families that were interested in participating in the study were asked to complete the survey and mail it back to the laboratory.

The infants who participated in this study were equally distributed across gender. Approximately 78% of the participants were Caucasian, 3% Asian, 3% Hispanic, and 16% of the participants had parents belonging to more than one ethnic group. All participants were born within two weeks of their due date, and none of them experienced birth complications, serious illness, or had a serious neurological disorder.

Procedure

Upon arrival at the laboratory, the parent and the infant were ushered into a testing room. There was a short warm-up session during which the infant became familiar with the room and the experimenter, while the parent received general explanations about the procedures. Parents were also informed that participation was voluntary, and that they could withdraw from the experiment at any time, for example if they felt that their infant was distressed. After the parent expressed his/her willingness to participate, s/he signed a consent form (see Appendix C).

Next, the parent was asked to sit in a chair with the infant on his/her lap. Startle blink EMG responses were measured from the activity of the *orbicularis oculi* muscle. To allow this recording, two miniature Ag/AgCl electrodes (6 mm diameter), filled with a standard conductive electrode cream, were attached to the skin beneath the infant's right eye. One electrode was placed directly below the pupil over the *orbicularis oculi* muscle and the other electrode was placed 1 cm laterally, to the right of the first electrode. In addition, two disposable electrodes were placed on the infant's back in order to record the electrocardiogram (ECG) during the emotion-modulated startle paradigm. A ground electrode was attached to the back of the infant's neck. Prior to the start of the experiment, the parent was given earplugs and a visor to cover his/her eyes. The purpose of these actions was to avoid a situation in which the parent's reactions to the foreground stimuli and the startle probes may bias the reactions of the infant during the experiment.

A computer monitor (16" viewable) was placed 1.5 m from the infant. The infant had a full view of the screen, but the space to the sides of the monitor and behind it was hidden by a large piece of plywood with a window in its center through which the monitor screen was exposed. By placing the plywood around the computer monitor, and dimming the room lights, it was hoped to minimize visual distractions and thus to attract infants' attention to the pictures displayed on the screen. In addition, the plywood allowed the experimenter to stand behind the monitor without being seen by the infant. A small hole in the plywood enabled the experimenter to observe the infant throughout the experiment. This was done to fulfill several functions: First, it was important to ensure that the EMG electrodes remained on the infant's face. If the electrodes fell off or were pulled off by the infant, the experimenter paused the experiment until the electrodes were properly reattached. Second, it was essential to mark trials during which the infant's attention was diverted from the picture presented on the monitor. If the infant was not looking at the picture by the time the startle probe was presented, the experimenter registered a button press that caused the stimulus presentation software to mark the trial as invalid. Using this information, such trials were later omitted from analysis. In addition, for approximately one third of the participants, the experimenter used a stopwatch to document the duration of looking time at each of the pictures. This was done in order to enable the examination of potential differences in looking time between the three experimental conditions. Third, if the infant became fussy or drowsy, or was not looking toward the monitor, the experimenter attempted to attract his/her attention by tapping rapidly on top of the monitor with her fingers. This act, if it occurred, took place toward the end of an intertrial interval or immediately following the onset of the foreground stimulus. Testing was terminated if the infant showed continuous signs of distress and/or upon maternal request.

The experiment included 30 stimuli arranged in 10 blocks of 3 trials. In each block there was one pleasant stimulus, one neutral stimulus, and one unpleasant stimulus, randomly ordered. It was assumed that a total of 30 trials would ensure a sufficient number of startle responses for each participant to enable a reliable comparison of responses across the three experimental conditions. Within each of the 3 trials, the face image was displayed for a duration of 5 s. This duration was thought to be long enough to allow an emotion-modulated effect to take place, yet it was relatively short in order to diminish habituation due to carry-over effects. Trials were separated by intervals varying from 15 to 20 s (offset to onset), the duration of which was also designed to reduce habituation. The blink-eliciting probe was presented 2.5 s after stimulus onset on 15 randomly-chosen trials and 4 s after stimulus onset on the remaining 15 trials. This was done in order to prevent predictability of the startle probe. The overall length of this experiment was approximately 10 min. The presentation of the startle probes and the foreground stimuli were controlled by the STIM Stimulus Presentation System (James Long Company, Caroga Lake, NY).

Stimuli

The startle probe. The acoustic startle probe consisted of a 100 dB peak SPL (A-scale) burst of white noise with instantaneous rise time presented binaurally for a duration of 50 ms. The probes were delivered through two wall-mounted speakers positioned 1.5 m on either side of the infant's ears. Several safety mechanisms were used to ensure that the sound level did not exceed 100 dB SPL. First, the sound level at the infant's ear was calibrated to 100 dB using an SPL meter. Second, the precise location of the speakers was fixed to avoid any deviation from the specified sound level that may occur throughout the course of the study. Third, the acoustic probe was generated by high-precision stimulus presentation software and hardware, and all stimulus properties were defined prior to stimulus presentation. Consequently, when triggered, only an acoustic probe of the pre-specified properties (100 dB SPL, 50 ms white noise) was presented. Finally, the sound level of the startle probe was tested on a regular basis to ensure that it remained the same for the entire duration of the study.

The choice of 100 dB peak SPL was based on an extensive literature review of startle research with infants and children, in which this or a higher dB level has been used over the last two decades and was reported to be safe, not overly aversive, and suitable for reliably producing startle in infants and children (e.g., Anthony & Graham, 1985; Balaban, 1995; Grillon, Dierker, & Merikangas, 1997; Hirano et al., 1996; Ornitz, Russell, Yuan, & Liu, 1996; Richards, 2000; Waters, Lipp, & Cobham, 2000).

<u>The foreground stimuli.</u> The foreground stimuli were pleasant, neutral, and unpleasant pictures displayed on a computer monitor. The pleasant stimulus was a photograph of an adult female posing a happy facial expression. The neutral stimulus was a photograph of a different adult female posing a neutral facial expression. The unpleasant stimulus was a photograph of a third adult female posing an angry facial expression. The neutral stimulus was included to serve as a comparison for the emotion-modulated effect.

All the photographs were taken from the NimStim Face Stimulus Set (Tottenham, Borscheid, Ellertsen, Marcus, & Nelson, 2002). Within this stimulus set, the physical dimensions and other characteristics of the photographs are standardized, and all the photographs are taken against a standard background. The sample of the stimulus set that was used in the current study was of three young Caucasian female models, each wearing a gray scarf to minimize differences in colors of clothing

Given that the three models were equally unfamiliar to the infant, and that many of the characteristics of the photos were standardized, it was assumed that differences in the startle response to each of the three stimulus types would likely be due to the different emotional displays. To control for the possible effect of differences between the models other than in their emotional display, the stimulus set used in this study included each of the three models posing all the three types of facial expressions (see Appendix D). Each infant was presented with one of three possible combinations of the three models, each posing a different emotional expression.

Data reduction and analysis

Startle response. Startle blink EMG responses to each of the acoustic probes were quantified using the following methodology. During recording, the raw EMG signal was amplified using a custom bioelectric amplifier (SA Instruments, San Diego, CA) with a gain of 1000 and with filter settings at 1 Hz (high pass) and 1250 Hz (low pass). The amplified signal was digitized on line with a 12-bit analogue-to-digital converter (full scale input range of 5V) at a sampling rate of 5000 Hz, using Snap-Master data acquisition software (HEM Data Crop). The high sampling rate was chosen in order to appropriately address two potential problems with regard to EMG startle analysis: First, to capture the full range of frequencies in the signal, the sampling rate has to be at least twice the Nyquist frequency, which is the maximum frequency that will be resolved in the signal (Stern, Ray, & Quigley, 2001). Second, in order to avoid aliasing, a situation in which activity at a frequency higher than the Nyquist frequency is randomly sampled, the low-pass filter setting on the bioamplifier has to be set to around half the Nyquist frequency. Given that the EMG signal contains activity up to 500 Hz, the most suitable setting for the low-pass filter on the bioamplifier was 1250 Hz (the next lowest being 250 Hz, which would have attenuated any EMG power between 250 and 500 Hz). Based on this setting, the optimal sampling rate was conservatively determined to be 5000 Hz to give a Nyquist frequency of 2500 Hz.

Once the EMG signal was collected, it was processed and analyzed using the EMG Analysis System from James Long Company (Caroga Lake, NY). First, the signal was digitally filtered offline between 28 and 500 Hz, following Van Boxtel, Boelhouwer, and Bos's (1998) recommendation for optimal analysis of acoustic blink reflexes. A digital band-stop filter (50-70 Hz) aimed at removing 60 Hz noise in the recorded signal was then applied to the data. Next, the signal was rectified and smoothed to make it more amenable to identification and scoring of the peak startle response. Rectification involved inverting negative values of the signal and combining them with the positive portion of the signal. The resulting signal was then smoothed (i.e., low-pass filtered) by passing a moving window of 20 ms duration over the data series in 2 ms increments. The processes of filtering, rectifying, and smoothing the raw EMG signal are illustrated in Figure 1. Once the raw signal was rectified and smoothed, participants' responses were scored for reflex size and response latency, based on which response probability and habituation of response were also calculated. Baseline activity of the *orbicularis oculi* muscle was also calculated to determine whether the face stimuli influenced the levels of tension in this muscle prior to blink onset (Bradley et al., 1991). This was done by averaging the rectified and smoothed EMG activity in each non-rejected trial during the initial 20 ms following the probe onset.

Based on inspection of the data, the time window during which startle blinks were considered was determined as 50-160 ms post startle probe onset. In order to be quantified, the peak of the reflex blink had to occur within this time window. The peak value of the reflex blink was computed relative to a baseline that extended from 50 ms pre-probe to probe onset. If EMG activity in the pre-probe baseline exceeded a threshold of 8 μV, the corresponding trial was rejected from further analysis. This threshold was established by inspection of the raw data. Trials were also rejected if the participant was looking away from the computer monitor at the time the foreground stimulus was presented.

For non-rejected trials, mean reflex size was computed in two ways: Peak magnitude and peak amplitude (see Berg & Balaban, 1999). The term "peak magnitude" refers to the mean size of the peak startle response when all responses are averaged over all trials in each experimental condition, including values of zero for trials without detectable responses. The term "peak amplitude" refers to the mean size of the startle response if the responses are averaged for trials with non-zero responses only. In other words, a magnitude analysis represents an average of peak EMG activity within a specified peak response window, regardless of whether the EMG activity is a startle response. An amplitude analysis, in contrast, averages peak EMG activity only for trials that were accepted as involving true startle reactions. For amplitude editing, the EMG signal was viewed graphically, and any trial that did not include a startle response in the pre-determined response time window was marked as missing data (for a similar procedure see Klorman, Cicchetti, Thatcher, & Ison, 2003). Inter-rater reliability was calculated to ensure that this editing process was consistent across editors. Next, peak magnitude and amplitude were calculated separately for each individual for each of the experimental conditions (pleasant, neutral, and unpleasant trials). To examine whether differences in reflex size existed between the experimental conditions, two repeated-measures analyses of variance (repeated-measures ANOVAs) were performed, with peak magnitude or amplitude for each experimental condition as the within-subjects factor. Only participants with at least two non-rejected trials in each experimental condition were included in this analysis. An additional repeated-measures ANOVA was performed with mean *orbicularis oculi* muscle baseline activity in each experimental condition as the within-subjects factor, in order to examine differences between conditions in the effect of the face stimuli on baseline EMG prior to the onset of the startle response.

Response latency was quantified as the time between startle probe onset and the time at which the startle peak occurred. Latency for peak magnitude and latency for peak amplitude were calculated separately for each individual for each of the experimental conditions. To examine whether differences in response latency existed between the experimental conditions, two repeated-measures ANOVAs were performed, with latency to peak magnitude or amplitude for each experimental condition as within-subjects factors.

To quantify response probability, the proportion of trials in which actual startle responses occurred was calculated out of the overall number of non-rejected trials. This calculation was performed as a whole for the entire number of trials, as well as separately for each experimental condition.

Habituation of the startle response was computed separately for peak magnitude and peak amplitude as the progressive decrease in reflex size as a function of repeated elicitation of startle probes. To analyze habituation, two one-way ANOVAs were performed with experimental block (i.e., order of startle probe presentation) as an independent factor and peak magnitude or amplitude as the dependent variable.

Theoretically, more statistical power could have been gained if a repeated-measures ANOVA was used to compute habituation of response, but this test was not possible since a large proportion of the participants were missing values of the dependent variable for at least one of the experimental blocks.

Heart period. The following methodology was used to quantify heart period (HP) during the experimental procedure. The ECG signal was amplified by a custom bioamplifier from SA Instruments, using a high-pass filter setting of 1 Hz, a low-pass setting of 1250 Hz, and a gain of 250. The signal was digitized alongside the EMG channel at 5000 Hz using Snap-Master data acquisition software (HEM Data Corp.). All subsequent processing and analysis of the ECG signal was carried out using the IBI Analysis System from James Long Company (Caroga Lake, NY). Firstly, R-wave detection was carried out offline using a 4-pass self-scaling peak detection algorithm. This gave a file containing the onset times of each detected R-wave in the physiological record. For artifact editing, the sampled ECG signal was viewed graphically alongside tick marks representing the times of software-detected R-waves. In the case of an obscured R-wave that was not detected by the software, a tick mark was inserted into the graphical ECG record. If the undetected R-wave was visible in the ECG, it was marked manually. If the R-wave was not visible, the tick mark was placed based on the specific editing rules of Byrne and Porges (1993). If a succession of R-waves were not visible due to excessive artifact, that specific section of the physiological record was marked as artifact and was not used in further analysis of HP.

The edited R-wave series was converted to a prorated HP series with an equal time interval of 250 ms between each prorated HP. Heart periods spanning two sampling intervals were prorated between these two intervals using a weighted-mean algorithm. Mean HP was then calculated from the prorated HP series.

For each participant, HP values were calculated for each 1-s interval across the time window from 5 s pre-picture presentation to 10 s post-picture presentation. Next, the difference in milliseconds between post-picture HP and pre-picture HP was calculated separately for each experimental condition. A repeated-measures ANOVA was then used to examine whether differences existed between the experimental conditions in the magnitude of change in HP exhibited following the presentation of the affective foreground stimuli. Only participants with at least two non-artifacted trials in each experimental condition were included in this analysis.

Looking time. Looking time was computed as the length of time a participant was looking at the computer monitor from the onset to the offset of each picture presentation. Using a stopwatch, an experimenter recorded on-line the precise time during which participants were looking at the monitor screen for each of the experimental trials. Given that the experimenter was standing behind the monitor, he was blind to the affective valence of each picture, and could only determine the onset and offset of its presentation based on the light coming out of the monitor. If participants were looking away from the monitor for the entire time of stimulus presentation, the trial was marked as a bad trial and was regarded as missing data. Mean looking time was then calculated for each participant for each of the experimental conditions. To examine whether differences in looking time existed between the experimental conditions, a repeated-measures ANOVA was performed, with mean looking time for each experimental condition as a within-subjects factor. Here, as well, a minimum of two non-rejected trials in each experimental condition was required in order for a participant to be included in the ANOVA.

General statistical assumptions and adjustments. An important assumption of ANOVA is that the dependent variable is distributed normally. Otherwise, any significant deviation from a normal distribution should be corrected using a suitable transformation, such as the natural logarithm transformation, which is commonly used with physiological data. Hence, prior to performing any of the ANOVAs reported in the text, two steps were taken to ensure normality. First, across all trials of the experiment, values of the dependent variables were screened for extreme scores both within and across participants. As commonly defined in the literature, outliers were classified as 3 SD above or below the mean for EMG data and 2 SD above or below the mean for all other data. Outlier scores were then rejected and marked as missing data. Second, the Kolmogorov-Smirnov test was used to compare the distribution of the raw data to a normal distribution. This test indicated that all the dependent variables described above had distributions that did not differ significantly from normality, and therefore no transformation was necessary.

The probability values of the ANOVAs described in the text have been adjusted using the Greenhouse-Geisser estimate of epsilon, which accounts for the correlation between repeated trials on the same individual (Geisser & Greenhouse, 1958).

Research hypotheses

Startle response. It was hypothesized that the size of the startle blink EMG response would vary according to the affective valence of the foreground stimulus. In line with the emotion-modulated startle paradigm, it was expected that relative to the peak startle response during the presentation of neutral stimuli, the startle response would be potentiated when the probe is presented during unpleasant foreground stimuli, and inhibited during pleasant foreground stimuli. In contrast, it was hypothesized that if attentional processes are salient enough to overcome the emotional effect of the foreground stimuli, the opposite pattern of modulation would be found. That is, in accordance with theories of startle modulation by attention, it was expected that relative to the peak startle response during the presentation of neutral stimuli, the startle response would be inhibited when the acoustic probe is presented during the less familiar, and therefore more attention holding, unpleasant visual foreground stimuli. The startle response would be potentiated during the more familiar, less attention holding, pleasant foreground stimuli. As for differences between the experimental conditions in the activity of the *orbicularis oculi* muscle prior to the onset of the startle response, it was hypothesized that no differences would be found between the presumably equally arousing pleasant and the unpleasant conditions. However, baseline EMG activity during these two conditions would be expected to be higher than during the less arousing neutral condition. The latency to the peak startle response was also hypothesized to vary according to the affective valence of the foreground stimulus. It was expected that relative to the latency to the peak startle response during the presentation of neutral stimuli, the peak latency would be faster (earlier) when the probe is presented during unpleasant foreground stimuli, and slower (later) during pleasant foreground stimuli. With respect to the habituation of the startle response, it was hypothesized that the intensity of the eyeblink startle reflex would diminish with repeated elicitations of acoustic startle probes. No specific hypothesis was made concerning the frequency of the startle response.

Heart period. In line with previous findings, it was hypothesized that participants' HR would vary according to the affective valence of the foreground stimulus. Specifically, it was expected that relative to infants' HR during the presentation of neutral stimuli, HR deceleration would be exhibited during the less familiar, more attention holding, unpleasant foreground stimuli, and HR acceleration would be exhibited during the more familiar, less attention holding, pleasant foreground stimuli. Given the inverse relation between HR and HP, the above hypotheses translate to an HP increase (HR deceleration) during unpleasant foreground stimuli, and an HP decrease (HR acceleration) during pleasant foreground stimuli.

Looking time. As with the previous hypothesis and the assumption that attention might have a role in startle modulation, participants were expected to demonstrate a differential looking time in response to the various foreground stimuli. Specifically, it was hypothesized that participants would look longer at the more novel, unpleasant stimuli than at the familiar pleasant and neutral stimuli.

Results

Startle response

Reflex size. Differences in reflex size between the experimental conditions were examined separately for peak magnitude and for peak amplitude of infants' startle blink EMG responses. Thirty out of the 32 infants who participated in the study were included in the analysis of peak magnitude, having at least two non-rejected trials in each experimental condition. The remaining two infants were excluded from this analysis due to an inability to place EMG electrodes beneath the infant's lower eyelid (n = 1) or excessive artifact due to general fussiness accompanied by extensive movements (n = 1). For the infants who participated in the peak magnitude analysis, the mean number of non-rejected trials during the viewing of happy, neutral, and angry faces was 5.83 (*SD* = 2.48), 6.03 (*SD* = 2.41), and 5.70 (*SD* = 2.73), respectively. Mean peak magnitude for each of these conditions was 20.42 μV (*SD* = 17.92), 22.68 μV (*SD* = 20.55), and 19.12 μV (*SD* = 19.27), respectively. A repeated-measures ANOVA indicated that the difference in peak magnitude between the three experimental conditions was not statistically significant, $F\ (2, 58) = 1.74$, *ns*, $\in = .69$.

To examine differences in peak amplitude between the experimental conditions, the EMG signal was viewed graphically, and any trial that did not include a startle response was marked as missing data. To establish reliability for this coding, the EMG responses of all participants were coded independently by the author and an undergraduate research assistant who was trained to determine whether a startle response occurred. Cohen's Kappa indicated a high inter-rater reliability between the author and the second coder, $K = .96$. Ten out of the 30 infants who were included in the peak magnitude analysis had less than two trials with a true startle reaction in at least one of the experimental conditions. These participants were excluded from the analysis of differences in peak amplitude between conditions. For the remaining 20 infants who comprised the sample for peak amplitude analysis, the mean number of accepted trials during the viewing of happy, neutral, and angry faces was 3.43 (*SD* = 2.47), 3.43 (*SD* = 2.39), and 3.20 (*SD* = 2.75), respectively. Mean peak amplitude for each of these conditions was 27.59 μV (*SD* = 17.48), 25.82 μV (*SD* = 18.21), and 23.70 μV (*SD* = 17.54), respectively. The results of a repeated-measures ANOVA yielded a significant difference in peak amplitude between the experimental conditions, F (2, 38) = 4.46, p < .05, ϵ = .86, η^2 = .19. Tests of within-subjects contrasts indicated that the differences in peak amplitude between the happy and the neutral conditions as well as between the angry and the neutral conditions were non-significant, F (1, 19) = 3.07, p = .10, and F (1, 19) = 2.28, *ns*, respectively. However, peak amplitude elicited during the happy condition was found to be significantly larger than peak amplitude elicited during the angry condition, F (1, 19) = 7.16, p < .05. Figure 2 illustrates the difference in peak amplitude between infants' startle responses elicited during the viewing of happy, neutral, and angry facial expressions.

Baseline EMG activity. Differences between conditions in *orbicularis oculi* muscle EMG activity prior to the onset of the startle response were examined for all non-rejected trials. Mean baseline EMG during the viewing of happy, neutral, and angry faces was 6.42 μV (*SD* = 7.37), 10.61 μV (*SD* = 16.49), and 5.82 μV (*SD* = 4.66), respectively. A repeated-measures ANOVA indicated a non-significant difference in *orbicularis oculi* muscle EMG activity between the experimental conditions, F (2, 58) = 1.84, *ns*, ϵ = .65.

Response latency. Differences in response latency between the experimental conditions were also examined separately for peak magnitude and for peak amplitude of infants' startle blink EMG responses. Mean latency to peak magnitude during the viewing of happy, neutral, and angry faces was 93.79 ms (*SD* = 15.94), 97.05 ms (*SD* = 14.62), and 93.67 ms (*SD* = 16.62), respectively. A repeated-measures ANOVA indicated a non-significant difference in latency to peak magnitude between the experimental conditions, F (2, 58) = 1.04, *ns*, $\epsilon = .92$. Mean latency to peak amplitude during the viewing of happy, neutral, and angry faces was 96.93 ms (SD = 16.81), 97.87 ms (SD = 15.38), and 98.54 ms (SD = 17.91), respectively. Here as well, a repeated-measures ANOVA showed that the difference between the experimental conditions was non-significant, F (2, 38) = .11, *ns*, $\epsilon = .85$.

Response probability. Table 1 shows the mean number of trials that were accepted for the analyses of peak magnitude and peak amplitude of infants' startle blink EMG responses, both as a whole for the entire number of trials and separately for each of the experimental conditions. As shown in Table 1, for the 30 infants who were included in the analysis of peak magnitude, the overall response probability was .58 (SD = .31), meaning that an actual startle response was present in approximately 58% of the non-rejected trials. The response probability during the viewing of happy, neutral, and angry faces was .59 (SD = .32), .57 (SD = .31), and .58 (SD = .38), respectively. A repeated-measures ANOVA with response probability for each experimental condition as a within-subjects factor showed that response probability did not vary as a function of the affective valence of the picture being presented, F (2, 58) = .10, *ns*, $\epsilon = .89$.

Habituation of response. To examine habituation in infants' startle response, two one-way ANOVAs were performed with experimental block (i.e., order of startle probe presentation: 1-6, 7-12, 13-18, 19-24, and 25-30) as an independent factor and peak magnitude/ amplitude as a dependent variable. With peak magnitude as a dependent variable, the ANOVA indicated a significant main effect of experimental block on infants' habituation of response, F (4, 25) = 10.06, $p < .001$, $\eta^2 = .62$. Habituation in startle magnitude occurred incrementally by block i.e., with repeated startle probe presentation. Similar results were obtained with peak amplitude as a dependent variable, F (4, 25) = 4.12, $p < .05$, $\eta^2 = .40$, indicating a significant decrease in the amplitude of infants' startle responses along with the repetition of startle probe presentation. Figure 3 illustrates the habituation in reflex size across experimental blocks for both peak magnitude and peak amplitude of infants' startle responses.

Heart period

Differences in HP between the three experimental conditions were examined using a repeated-measures ANOVA. The within-subjects factor was the difference in milliseconds between post-picture HP and pre-picture HP. The magnitude of change in HP following the presentation of the happy, neutral, and angry faces was 10.09 ms (*SD* = 13.53), 9.65 ms (*SD* = 12.23), and 14.34 ms (*SD* = 12.76), respectively. The ANOVA indicated that the change in HP following the presentation of the pictures varied as a function of the affective valence of the picture, F (2, 62) = 2.44, $p < .10$, $\epsilon = 1.00$, $\eta^2 = .07$. While this result was non-significant at the .05 alpha level, follow-up contrasts showed that the magnitude of change in HP exhibited following the presentation of angry faces was significantly larger than the mean magnitude of change in HP exhibited following the presentation of happy and neutral faces, F (1, 31) = 5.01, $p < .05$. These findings are illustrated in Figure 4.

Looking time

Looking time data were collected from 12 participants. The mean length of time during which infants were looking at the happy, neutral, and angry faces was 3.65 s (*SD* = .65), 3.82 s (*SD* = .62), and 4.17 s (*SD* = .39), respectively. A repeated-measures ANOVA was performed with mean looking time for each experimental condition as a within-subjects factor. The results of this analysis indicated a difference in looking time between the three experimental conditions, $F\ (2, 22) = 2.84$, $p < .10$, $\epsilon = .84$, $\eta^2 = .21$. These results were non-significant at the .05 alpha level, however follow-up contrasts showed that the length of time during which participants were looking at the angry faces was significantly longer than the length of time during which they were looking at the happy faces, $F\ (1, 11) = 5.19$, $p < .05$. The difference in looking time between the experimental conditions is illustrated in Figure 5.

Discussion

The purpose of this research project was to investigate whether the startle reflex of 9-month-old infants can be modulated by emotional stimuli. As well, this study was aimed at examining the specific characteristics of infants' startle response, including the intensity of and the latency to the peak response, the frequency of its occurrence in response to acoustic probes, and the degree of habituation in reflex size as a function of repeated presentation of the startle probe. To facilitate the understanding of the role of attention in infants' startle modulation, this study also incorporated the measurements of autonomic reactivity and looking time during the emotion-modulated startle paradigm.

The results indicated that with the stimulus set utilized in this study, 9-month-old infants do not demonstrate a pattern of emotion-modulated startle effect that is similar to the one found in adults. While adults typically show potentiation of startle blink EMG response during the viewing of unpleasant pictures and inhibited response during the viewing of pleasant pictures, an opposite effect was obtained in the current study. In comparison to blink amplitude evoked in response to acoustic probes elicited during the presentation of neutral facial expressions, infants' startle responses were potentiated during the presentation of happy facial expressions, and inhibited during the presentation of angry facial expressions. Importantly, these differences cannot be attributed to the influence of the face stimuli on levels of EMG activity prior to blink onset, as no differences were found between conditions in baseline *orbicularis oculi* muscle activity. Latency to peak response did not differ as a function of the affective valence of the picture displayed at the time that the acoustic probe was presented.

One possible understanding of the opposite pattern of startle modulation found in the current study compared with the adult work is that when presented with generally similar emotional foreground stimuli (i.e., pleasant and unpleasant pictures), infants and adults demonstrate an opposite pattern of emotion-modulated startle reaction. On theoretical grounds it is difficult to support such an interpretation, as it fundamentally contradicts the motivational mechanism accounting for the emotional modulation of the startle reflex. According to Lang and his colleagues (for reviews see Bradley et al., 1999; Lang, 1995; Lang et al., 1990, 1992, 1997b, 1998), the protective-defensive startle reflex is expected to be greater when there is a match between the organism's ongoing state and the defensive motivational system activated by the startle-inducing stimulus. Thus, evoking the defensive startle reflex in the context of an ongoing defensive motivational state (i.e., when the individual is reacting to an unpleasant foreground stimulus) results in a reflex-affect match, which supposedly prompts a reflex response that is of significantly greater size. The same evoked defensive reflex is expected to be reduced in size when the organism is processing an appetitive stimulus (i.e., is in states of pleasure), hence a mismatch exists between its affect and the nature of the startle reflex. The motivational priming mechanism has gained considerable support from research with adults (Bradley et al., 1990, 1993, 2001; Bradley & Lang, 2000; Dichter et al., 2002; Ehrlichman et al., 1995; Jansen & Frijda, 1994; Kaviani et al., 1998, 1999; Miltner et al., 1994; Sabatinelli et al., 2001; Vrana et al., 1988), therefore the fact that an exactly opposite effect was obtained with 9-month-old infants is puzzling. In fact, as described earlier in the text, an adult-like pattern of emotion-modulated startle effect was found in Balaban's (1995) study with 5-month-old infants. Thus, on both theoretical and experimental grounds it is unclear why a replication of the emotional modulation of startle would be obtained with 5-month-old infants, while 9-month-old infants demonstrate an opposite effect.

Two alternative explanations ought to be considered with respect to the modulated startle response exhibited by 9-month-old infants in the present study. It is possible that young infants do not display an emotion-modulated startle effect, perhaps because their emotional world is not organized around appetitive and aversive motivational systems as in adults. Indeed, an attempt to replicate an emotional modulation of startle in school-age children also failed to demonstrate an adult-like pattern of modulation (McManis et al., 1995). Alternatively, it is also plausible that infants do show an emotion-modulated startle effect, but that the specific set of emotional foregrounds used in the present study failed to elicit the expected pattern of modulation. This might have been the case because face stimuli, in general, do not sufficiently elevate infants' levels of arousal. Indeed, high levels of arousal in adults are sometimes manifested by greater EMG activity prior to blink onset (Bradely et al., 1990; Cook et al., 1992), whereas in the present study, no differences between conditions were found in baseline *orbicularis oculi* muscle activity.

It is also possible that happy, neutral, and angry facial expressions do not induce pleasant, neutral, and unpleasant states (respectively) in 9-month-old infants. In particular, 9-month-old infants might not be sufficiently familiar with angry facial expressions to recognize and understand their meaning, and to elicit an appropriate response (Malatesta et al., 1986; Malatesta & Haviland, 1982). Although infants of this age discriminate between different expressions, such discrimination might be based on specific differences in facial features, such as the shape of the mouth (Caron et al., 1985; Kestenbaum & Nelson, 1990; Ludemann, 1991; Serrano et al., 1995) or on the degree of familiarity of the expression (Kagan, 1974; Ornitz, 1999), rather than on affective qualities. This lack of emotional meaning attached to the foreground stimuli might have allowed other factors, such as differentiated attention level, to influence the direction of modulation of the startle response (Lang et al., 1990). Since 9-month-old infants are more familiar with happy facial expressions than they are with angry facial expressions (Malatesta et al., 1986; Malatesta & Haviland, 1982), they might allocate more attention to the novel angry face and less attention to the familiar happy face. According to Graham's attention theory, these different levels of visual attention would modulate the effect of the acoustic probe by facilitating or reducing the degree of mismatch in sensory modality between the foregrounds and the probe (e.g., Anthony & Graham, 1983, 1985; Hackley & Graham, 1984). Specifically, Graham reported that both infants and adults demonstrate a smaller startle response when there is a sensory mismatch between the probe modality and the modality of the foreground stimulus (Anthony & Graham, 1985). This inhibition of response is interpreted in terms of limited attentional resources that are allocated *a priori* according to modality. Thus, when individuals engage in a task requiring modality-specific attention, fewer resources are available to the alternate modality through which the probe stimulus is presented, rendering an inhibited startle response. Given that more interesting foreground stimuli engage attention to a greater extent than do less interesting foreground stimuli, a greater cross-modality startle response inhibition is expected with interesting than with dull foregrounds (Anthony & Graham, 1983, 1985; Simons & Zelson, 1985). In the context of the present study, startle inhibition indeed occurred when the sensory mismatch was facilitated during the viewing of the relatively novel, more interesting and engaging angry facial expressions, whereas startle potentiation took place when the sensory mismatch was reduced during the viewing of the familiar, less attention-holding happy facial expressions. Thus, the current findings do not confirm the motivational priming mechanism for emotion-modulated startle in 9-month-olds, but instead are consistent with the sensory-selective account for startle modulation.

Measures of cardiac activity and looking time during each of the experimental conditions provided additional affirmation for the role of attention in infants' startle modulation. The cardiac data indicated that 9-month-old infants exhibit a significantly larger increase in HP (decrease in HR) when viewing angry faces than when viewing happy or neutral faces. Given the extensive literature associating decreased HR with increased attention (Graham et al., 1983; Lacey & Lacey, 1974; Porges, 1992), the HP findings obtained in this study support an increased attentional allocation to angry faces. Infants' behavior during stimuli presentation, as manifested in the duration of time spent looking at each type of foreground stimulus, also indicated greater attention allocation to angry faces than to happy faces.

Regardless of whether emotional modulation of startle was not obtained in the current study because of the specific set of emotional foregrounds that were used or because infants, in general, may not show emotion-modulated startle responses, the fact that Balaban (1995) did obtain the adult-like pattern of emotion-modulated startle in infants despite using a similar set of foreground stimuli (i.e., pictures of happy, neutral, and angry faces) needs to be addressed. It should be noted that the infants in Balaban's (1995) study were 5 months old, while the infants in the current study were 9 months old. While both studies add to the literature that infants in both these age groups can discriminate happy from angry faces depicted in photographs (Berrera & Maurer, 1981; Kestenbaum & Nelson, 1990; LaBarbera, Izard, Vietze, & Parisi, 1976; Ludemann & Nelson, 1988; Oster & Ewy, 1980; Schwartz, Izard, & Ansul, 1985; Serrano et al., 1992, 1995), differences between 5- and 9-month-old infants may exist in their interest in or preference for each of these expressions. For example, it is possible that 5-month-old infants allocate more attention to happy faces than angry faces, whereas 9-month-old infants prefer angry faces to happy faces. This might be due to developmental changes in infants' preferences for novelty or familiarity after repeated presentations of the face stimuli (see Hunter & Ames, 1988; Roder, Bushnell, & Sasseville, 2000). Indeed, studies have shown that in a visual preference test, 4-month-old infants look longer at joyful expressions than angry or neutral ones (LaBarbera et al., 1976), but at 7 months of age infants look longer at fearful than happy faces (de Haan & Nelson, 1998; Kotsoni, de Haan & Johnson, 2001; Nelson & Dolgin, 1985). As described earlier, Graham's attention theory relates such visual preferences to differential responses to an acoustic startle probe. Thus, allocating more visual attention to happy facial expressions than to angry facial expressions (as might have been the case with 5-month-old infants) would lead to inhibited startle blink EMG responses to acoustic probes elicited during the viewing of happy faces, and potentiated responses during the viewing of angry faces. In contrast, if visual attention is greater while viewing angry facial expressions than while viewing happy facial expressions (as demonstrated with 9-month-old infants), startle blink EMG responses would be inhibited to acoustic probes presented during the viewing of angry faces, and potentiated during the viewing of happy faces. This line of reasoning does not support the view that infants' startle responses were affectively mediated in both the current study and that of Balaban (1995). Instead, it is possible that in both studies, the observed modulation of the startle response results from attentional factors related to the relative familiarity or unfamiliarity of the facial expression (Kagan, 1974; Ornitz, 1999).

To further examine the effect of emotional foreground stimuli on 9-month-old infants' startle modulation, as well as to tease apart emotional and attentional explanations for startle modulation in infancy, a second study was designed utilizing a different set of foreground stimuli that were thought to be more emotionally salient for this age group. This study is described in the next chapter.

STUDY 2

Overview

In this follow-up study, 9-month-old infants were tested in a second, modified emotion-modulated startle paradigm that was especially designed to elicit prominent positive and negative affect in this age group. This paradigm included delivery of an acoustic startle probe while infants were engaged in affectively pleasant, neutral, and unpleasant activities. The activities were an enjoyable game of peek-a-boo with the experimenter, an affectively neutral presentation of a spinning bingo wheel containing colorful balls, and a mildly frustrating episode of arm restraint during which infants were prevented from playing with an attractive toy placed in front of them.

The purpose of this study was to further examine the effect of emotional foreground tasks on 9-month-old infants' startle modulation in an attempt to establish whether the emotional modulation of startle, as widely replicated in adult studies, is present in infancy. By utilizing emotionally salient activities as foregrounds, the present study was designed to explore whether the unexpected pattern of startle modulation obtained in Study 1 was attributable to the specific set of stimuli that were utilized in that study, or whether it indicates that regardless of the nature of the foregrounds, 9-month-old infants do not display the emotion-modulated startle effect in the same way as adults. The importance of this study is in its contribution to the understanding of psychological processes underlying startle modulation in infancy. As in Study 1, the current study incorporated the measurement of autonomic reactivity and behavioral responsiveness (i.e., facial expressions and attention allocation) in each experimental condition in order to facilitate the understanding of the role of emotion and attention in infants' startle modulation. In addition to the modulation of infants' startle response, the present study also further investigated the specific characteristics of infants' eyeblink reflex, including the parameters of intensity of and latency to the peak response, frequency of its occurrence, and levels of habituation.

Methods

Participants

Twenty five 9-month-old infants (± 7 days) participated in this study (13 males, 12 females). Three additional infants were seen at the laboratory, however neither EMG nor cardiac data could be obtained from these participants due to high levels of distress displayed by the infants (n = 2), or because of a technical problem with data collection (n = 1).

As in Study 1, the pool of participants was recruited through commercially available mailing lists. Approximately 56% of the participants were Caucasian, 8% Asian, 12% African-American, and 24% of the participants had parents belonging to more than one ethnic group. All participants were born within two weeks of their due date, and none of them experienced birth complications, serious illness, or had a serious neurological disorder.

Procedure

The steps that were taken to prepare for data collection, including the EMG and ECG electrode placement, were similar to the ones described in Study 1 (see Appendix E for the informed consent form used in Study 2). The experiment itself included 3 episodes: Pleasant, neutral, and unpleasant, during each of which 6 acoustic startle probes were administered. Based on the response probability in Study 1, it was assumed that a total number of 18 probes would ensure a sufficient number of startle responses for each participant to enable a reliable comparison of responses across the three experimental conditions. The pleasant and unpleasant episodes were modified from the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith & Rothbart, 1999), and were designed to elicit positive and negative affect (respectively) in infants and young children. The precise procedure involved in each episode is described below.

<u>A pleasant episode – A peek-a-boo game.</u> The infant was seated on the parent's lap and in front of them there was a large cardboard screen. An experimenter sat behind the cardboard and out of the infant's sight. The episode began as the experimenter asked the infant "__________ (infant's name), where's __________ (experimenter's name)?" After a pause of 3 s, the experimenter raised her head above the cardboard, smiled to the infant, and said playfully "peek-a-boo!" The experimenter stayed in sight for an additional 5 s, during which the acoustic startle probe was presented. The experimenter then lowered her head so it was out of the infant's sight again, waited 5 more s, and then repeated the same procedure 5 more times.

<u>A neutral episode – A bingo wheel.</u> The infant was seated on the parent's lap and an experimenter sat in front of them holding a bingo wheel containing one colorful ball. The episode began when the experimenter started spinning the bingo wheel. The wheel was spun for 15 s during which one startle probe was presented. After 15 s, the experimenter added two colorful balls to the bingo wheel and continued spinning it for

additional 15 s during which another startle probe was presented. The experimenter then added four more colorful balls to the bingo wheel and spun the wheel for additional 15 s, during which a third startle probe was presented. After a pause of 10 s, the experimenter took six balls out of the bingo wheel and repeated the same procedure one more time.

An unpleasant episode – Gentle arm restraint. Prior to the beginning of this episode, the parent was given the following instructions on when and how to hold down his/her infant's arms: "When the experimenter signals you, please place your hands gently on your baby's forearms, move them to his/her side and continue to hold them there gently yet firmly enough so that s/he cannot pull free for 30 s. At this point (or earlier if your baby becomes very distressed), you will receive another cue from the experimenter to release your baby's arms." The session began when the experimenter showed the infant, who was seated on the parent's lap, an attractive toy (e.g., a colorful rattle). The experimenter said to the infant: "Look at this beautiful toy. Would you and your dad/mom like to play with it for a little while?" and then demonstrated how to play with the toy. The experimenter gave the toy to the infant and let him/her play with it for 15-30 s, until the experimenter judged that the infant was engaged with the toy. At the appropriate time, the experimenter cued the parent (nodded her head) to restrain the infant's arms for 30 s. During this 30-s time interval, two startle probes were presented, 15-20 s apart. After 30 s (or less if the infant became very distressed), the parent was cued to release her infant's arms, and to continue playing with the toy. The same procedure was repeated two more times. To reduce any distress elicited during the episode, the infant was allowed to play with the toy at the end of the episode.

The three episodes were presented in a counterbalanced order to control for a potential order effect on the characteristics of the startle response. The presentation of the startle probes was controlled by software (STIM Stimulus Presentation System, James Long Company, Caroga Lake, NY). The overall length of the experimental procedure was approximately 10 min. As in Study 1, if participants displayed increased fussiness or crying, which indicated that they found the episode they were engaged in as very distressing or aversive, the experimenter paused the activity until the infant recovered from the distress. In addition, parents were informed that they could request to terminate the experiment at any time without any penalty. The experiment was videotaped in order to allow the examination of facial expressions associated with each experimental condition. This was important in order to investigate whether the peek-a-boo game, the bingo wheel, and the arm-restraint episode were indeed successful in eliciting the corresponding positive, neutral, and negative affective states in 9-month-old infants. The videotapes were also used to code infants' levels of attention during each experimental episode. This was done in order to examine the role of attention allocation in the modulation of the startle reflex.

Stimuli

The startle probe. See Study 1.

Data reduction and analysis

Startle response. See Study 1.

Heart period. Cardiac activity during the experimental procedure was quantified using the same methodology as in Study 1, with the following exception. For each participant, mean HP was computed for a time window of 5 s prior to each startle probe presentation, and was then averaged separately for each of the three experimental conditions. It was assumed that the 5 s before each startle probe presentation was a sufficiently long epoch to quantify the HP of the infant during the specific experimental episode prior to the presentation of the startle probe, yet not long enough to be contaminated by the effect of the preceding probe. A repeated-measures ANOVA was used to examine whether participants' HP prior to the presentation of the startle probe differed across the experimental conditions.

Facial expressions. Behavioral manifestations of emotion were coded from videotapes by an observer who was unaware of the purpose of the study. The observer was instructed to make a subjective judgment as to whether the infant displayed positive (+1), neutral (0), or negative (-1) facial expressions during the 5 s prior to each startle probe. It was assumed that the 5-s time interval before each startle probe presentation was sufficiently long to represent the affective state of the infant associated with the specific experimental episode prior to the presentation of the startle probe, but not long enough to be contaminated by the effect of the preceding probe. Mean facial expression score was then computed for each participant for each of the experimental episodes. To examine whether differences in emotional expressions existed between the experimental conditions, a repeated-measures ANOVA was performed, with mean facial expression score as the dependent variable and each experimental condition as the within-subjects factor.

Attention. The videotapes were also used to code infants' levels of attention allocation during each of the experimental conditions. As in the affect coding, the coding of attention allocation was done by an observer who was unaware of the purpose of the study. The observer was instructed to make a subjective judgment as to whether the infant demonstrated on-task behavior (+1) or off-task behavior (0) during the 5 s prior to each startle probe. Infants were coded as ‘on-task” if they looked at the experimenter or at the stimulus in front of them (e.g., bingo wheel), and were attentive to the activity of the particular episode. Infants were coded as “off-task” if they were looking away from the experimenter or the stimulus and were not attentive to the ongoing activity. Mean attention level was then computed for each participant for each of the experimental episodes. To examine whether differences in attention allocation existed between the experimental conditions, a repeated-measures ANOVA was performed, with mean attention score as the dependent variable and each experimental condition as the within-subjects factor.

Research hypotheses

Startle response. It was hypothesized that the size of the startle blink EMG response would vary according to the emotional valence of the episode during which the startle probe was presented. In line with the emotion-modulated startle paradigm, it was expected that relative to the peak startle response during the presentation of the bingo wheel, the startle response would be potentiated when the probe is presented during the episode of arm restraint, and inhibited during the peek-a-boo game. In contrast, it was hypothesized that if 9-month-old infants do not exhibit the emotion-modulated startle effect, no specific pattern of startle modulation would be found. As for differences between the experimental conditions in the activity of the *orbicularis oculi* muscle prior to the onset of the startle response, it was hypothesized that no differences would be found between the pleasant and the unpleasant conditions. However, baseline EMG activity during these two conditions would be expected to be higher than during the less arousing neutral condition. If emotional modulation of startle is manifested, the latency to the peak startle response was also hypothesized to vary according to the emotional valence of the episode during which the startle probe was presented. It was expected that relative to the latency to the peak startle response during the presentation of the bingo wheel, the peak latency would be faster (earlier) when the probe is presented during the episode of arm restraint, and slower (later) during the peek-a-boo game. With respect to the habituation of the startle response, it was hypothesized that the intensity of the peak startle response would diminish with the repeated elicitation of acoustic startle probes. The frequency of the startle response was expected to be similar to the one obtained in Study 1 (approximately 58%).

Heart period. Based on the findings obtained in previous studies of emotional modulation of startle and in Study 1, it was hypothesized that participants' HP would vary across experimental episodes according to their emotional valence. Specifically, it was expected that relative to infants' HP during the presentation of the bingo wheel, HP decrease (i.e., HR acceleration) would be exhibited during the arm restraint episode, and HP increase (i.e., HR deceleration) would be exhibited during the peek-a-boo game.

Facial expressions. Participants' facial expressions were expected to vary across experimental episodes according to their emotional valence. Specifically, it was hypothesized that infants would demonstrate a neutral facial expression during the presentation of the bingo wheel, more positive facial expressions during the peek-a-boo game, and more negative facial expressions during the episode of arm-restraint.

Attention. The pleasant, neutral, and unpleasant experimental episodes were designed to elicit the corresponding affect in infants, but they were not expected to attract differential levels of attention. Participants' levels of attention were therefore not expected to vary across experimental episodes.

Results

Startle response

Reflex size. Differences in reflex size between the experimental conditions were examined separately for peak magnitude and for peak amplitude of infants' startle blink EMG responses. Twenty-three out of the 25 infants who participated in the study had at least two non-rejected trials in each experimental condition and were included in the analysis of peak magnitude. The remaining two infants were excluded from this analysis because of excessive artifact due to general fussiness accompanied by extensive movements. For the infants who participated in the peak magnitude analysis, the mean number of non-rejected trials during the peek-a-boo, bingo, and arm-restraint episodes was 5.00 (*SD* = 1.17), 5.70 (*SD* = .56), and 4.91 (*SD* = 1.38), respectively. Mean peak magnitude for each of these conditions was 14.86 μV (*SD* = 9.83), 22.14 μV (*SD* = 10.45), and 21.04 μV (*SD* = 19.53), respectively. A repeated-measures ANOVA indicated that the difference in peak magnitude between the three experimental conditions was statistically significant, F (2, 44) = 3.63, $p < .05$, $\epsilon = .80$, $\eta^2 = .14$. Follow-up contrasts revealed that the peak magnitude elicited during the peek-a-boo condition was significantly smaller than the peak magnitude elicited during the arm restraint, F (1, 22) = 5.96, $p < .05$, as well as during the spinning bingo wheel, F (1, 22) = 8.43, $p < .05$. The difference between the bingo wheel and the arm restraint conditions was non-significant, F (1, 22) = .10, *ns*. Figure 6 illustrates the difference in peak magnitude between infants' startle responses elicited during the peek-a-boo, bingo, and arm-restraint episodes.

To examine differences in peak amplitude between the experimental conditions, the EMG signal was viewed graphically, and any trial that did not include a startle response was marked as missing data. To establish reliability for this coding, the EMG responses of 58% of the participants were coded independently by the author and an undergraduate research assistant, who was trained to determine whether a startle response occurred. Cohen's Kappa indicated a high inter-rater reliability between the author and the second coder, $K = .84$. Three out of the 23 infants who were included in the peak magnitude analysis had less than two trials with a visible startle response in at least one of the experimental conditions. These participants were excluded from the analysis of differences in peak amplitude between conditions. For the 20 infants who comprised the sample for peak amplitude analysis, the mean number of accepted trials during the peek-a-boo, bingo, and arm-restraint episodes was 2.91 (*SD* = 1.56), 5.17 (*SD* = .98), and 3.70 (*SD* = 1.85), respectively. Mean peak amplitude for each of these conditions was 18.10 µV (*SD* = 11.47), 23.47 µV (*SD* = 10.58), and 27.37 µV (*SD* = 19.98), respectively. The results of a repeated-measures ANOVA yielded a significant main effect for peak amplitude between the experimental conditions, F (2, 38) = 4.51, $p < .05$, $\epsilon = .74$, $\eta^2 = .19$. Within-subjects contrasts indicated that the differences in peak amplitude between the peek-a-boo and the bingo wheel conditions as well as between the arm restraint and the bingo wheel conditions were non-significant, F (1, 19) = 3.80, *ns*, and F (1, 19) = .99, *ns*, respectively. However, peak amplitude elicited during arm restraint was found to be significantly larger than peak amplitude elicited during peek-a-boo, F (1, 19) = 14.42, $p < .001$. Figure 7 illustrates the difference in peak amplitude between infants' startle responses elicited during the peek-a-boo, bingo, and arm-restraint episodes.

Baseline EMG activity. Differences between conditions in *orbicularis oculi* muscle EMG activity prior to the onset of the startle response were examined for all non-rejected trials. Mean baseline EMG activity during the peek-a-boo, bingo, and arm-restraint episodes was 6.64 μV (SD = 4.86), 3.84 μV (SD = 1.71), and 5.49 μV (SD = 3.32), respectively. A repeated-measures ANOVA indicated a statistically significant difference in *orbicularis oculi* muscle EMG activity between the experimental conditions, F (2, 44) = 4.69, $p < .05$, $\epsilon = .77$, $\eta^2 = .18$. Follow-up contrasts showed a non-significant difference in baseline EMG activity between the peek-a-boo and the arm restraint conditions, F (1, 22) = 2.39, *ns*. Baseline activity during the spinning bingo wheel was found to be significantly smaller in amplitude than baseline activity in both the peek-a-boo and the arm restraint conditions, F (1, 22) = 6.03, $p < .05$, and F (1, 22) = 3.99, $p < .05$, respectively. These differences in baseline EMG activity are illustrated in Figure 8.

Response latency. Differences in response latency between the experimental conditions were also examined separately for peak magnitude and for peak amplitude of infants' startle blink EMG responses. Mean latency to peak magnitude during the peek-a-boo, bingo, and arm-restraint episodes was 91.86 ms (SD = 13.86), 91.24 ms (SD =

14.55), and 93.26 ms (*SD* = 17.25), respectively. A repeated-measures ANOVA indicated a non-significant difference in latency to peak magnitude between the experimental conditions, F (2, 46) = .16, *ns*, $\epsilon = .99$. Mean latency to peak amplitude during the peek-a-boo, bingo, and arm-restraint episodes was 93.75 ms (*SD* = 14.20), 91.05 ms (*SD* = 15.96), and 93.24 ms (*SD* = 21.95), respectively. Here as well, a repeated-measures ANOVA showed that the difference between the experimental conditions was non-significant, F (2, 40) = .22, *ns*, $\epsilon = .95$.

Response probability. Table 2 shows the mean number of trials that were accepted for the analyses of peak magnitude and peak amplitude of infants' startle blink EMG responses, both as a whole for the entire number of trials and separately for each of the experimental conditions. As shown in Table 2, for the 23 infants who were included in the analysis of peak magnitude, the overall response probability was .75 (*SD* = .20), meaning that an actual startle response was present in 75% of the non-rejected trials. The response probability during the peek-a-boo, bingo, and arm-restraint episodes was .61 (*SD* = .30), .90 (*SD* = .13), and .72 (*SD* = .31), respectively. A repeated-measures ANOVA with response probability for each experimental condition as a within-subjects factor showed that response probability varied significantly as a function of the affective valence of the episode during which the startle probes were elicited, F (2, 44) = 14.29, $p < .001$, $\epsilon = .99$, $\eta^2 = .39$. Within-subjects contrasts indicated that response probability during the bingo episode was significantly higher than response probability during peek-a-boo, F (1, 22) = 26.31, $p < .001$, and arm restraint, F (1, 22) = 9.99, $p < .05$. In addition, response probability during arm restraint was significantly higher than the response probability during the peek-a-boo episode, F (1, 22) = 4.87, $p < .05$.

Habituation of response. To examine habituation in infants' startle response, two one-way ANOVAs were performed with experimental block (i.e., order of startle probe presentation: Trials 1-6, 7-12, and 13-18) as an independent factor and peak magnitude/ amplitude as a dependent variable. With peak magnitude as a dependent variable, the ANOVA indicated a non-significant main effect of experimental block on infants' habituation of response, F (2, 15) = 1.18, *ns*. Similar results were obtained with peak amplitude as a dependent variable, F (2, 15) = 1.21, *ns*.

Heart period

Differences in HP between the three experimental conditions were examined using a repeated-measures ANOVA. The within-subjects factor was mean HP in milliseconds prior to startle probes onset. Mean HP across trials for the peek-a-boo, bingo, and arm-restraint episodes was 476.56 ms (*SD* = 39.67), 480.76 ms (*SD* = 39.80), and 450.86 ms (*SD* = 30.65), respectively. The ANOVA indicated that mean HP varied as a function of the affective valence of the experimental episode, F (2, 46) = 8.39, $p <$ .001, $\in = .87$, $\eta^2 = .27$. Follow-up contrasts showed that mean HP during arm restraint was significantly smaller than mean HP during the peek-a-boo episode, F (1, 23) = 12.79, $p < .05$, and the bingo wheel episode, F (1, 23) = 10.33, $p < .05$. The difference in HP between the peek-a-boo and the bingo wheel episodes was non-significant, F (1, 23) = .36, *ns*. These findings are illustrated in Figure 9.

Facial expressions

Participants' facial expression during each experimental trial was coded as either positive, neutral, or negative, prior to the onset of each startle probe. To establish reliability for this coding, 70% of the sample was coded independently by two undergraduate research assistants, who were trained to determine participants' affect. Cohen's Kappa indicated a moderately high inter-rater reliability between the two coders, *K* = .74. Mean affect during the peek-a-boo, bingo, and arm-restraint episodes was .48 (*SD* = .38), .02 (*SD* = .15), and –.37 (*SD* = .32), respectively. A repeated-measures ANOVA was performed, with mean affective display for each experimental condition as a within-subjects factor. The results of this analysis indicated a significant main effect of experimental condition, F (2, 46) = 45.89, $p < .001$, $\epsilon = .81$, $\eta^2 = .66$. Follow-up contrasts showed that significantly more positive affective facial displays were exhibited during the peek-a-boo condition than for both the spinning bingo wheel, F (1, 23) = 30.74, $p < .001$, and the arm restraint conditions, F (1, 23) = 62.22, $p < .001$, and that significantly more negative affective displays were exhibited during arm restraint than during the bingo wheel condition, F (1, 23) = 29.38, $p < .001$. Mean affective display during the experimental conditions is illustrated in Figure 10.

Attention

Participants' attention during each experimental trial prior to the onset of each startle probe was coded as either on-task or off-task. Mean attention level during the peek-a-boo, bingo, and arm-restraint episodes was .86 (*SD* = .19), 1.00 (*SD* = .00), and .91 (*SD* = .16), respectively. A repeated-measures ANOVA was performed, with mean attention level for each experimental condition as a within-subjects factor. The results of this analysis indicated a significant main effect of experimental condition, F (2, 46) = 5.50, $p < .05$, $\epsilon = .73$, $\eta^2 = .19$. Follow-up contrast analyses showed that significantly more on-task behaviors were displayed during the spinning bingo wheel episode than during the peek-a-boo, F (1, 23) = 12.94, $p < .05$, and the arm restraint episodes, F (1, 23) = 8.46, $p < .05$. Mean attention level during the experimental conditions is illustrated in Figure 11.

Discussion

The main purpose of Study 2 was to follow up the results obtained in the first study by examining whether age-appropriate and affectively salient foreground tasks elicit a pattern of startle modulation in 9-month-old infants that is similar to that established in adults. To further explore the unique role of attention and emotion in infants' startle modulation, this study also incorporated the measurements of autonomic reactivity and facial expressions during each of the experimental conditions.

The results revealed that in comparison to infants' startle blink responses to startle probes elicited during an affectively neutral bingo wheel episode, startle potentiation was exhibited during an affectively unpleasant activity (arm restraint), whereas an inhibited startle response was manifested during an affectively pleasant game (peek-a-boo). This pattern of startle modulation is similar to that documented with adults using the IAPS and other affectively salient foregrounds (Bradley et al., 1990, 1993, 2001; Bradley & Lang, 2000; Dichter et al., 2002; Ehrlichman et al., 1995; Jansen & Frijda, 1994; Kaviani et al., 1998, 1999; Miltner et al., 1994; Sabatinelli et al., 2001; Vrana et al., 1988).

Furthermore, as in adults, greater baseline activity of the *orbicularis oculi* muscle prior to blink onset was found during both pleasant and unpleasant conditions, relative to the neutral condition – a pattern found in adult work and ascribed to arousal processes (Bradley et al., 1990; Cook et al., 1992). Importantly, startle modulation cannot be attributed to these differences in baseline EMG, as *orbicularis oculi* activity prior to blink onset did not differ significantly between the pleasant and the unpleasant conditions. However, unlike the typical adults' emotional modulation of startle, latency to peak response did not differ as a function of the affective valence of the foreground task. Also, the repetition of startle probe presentation did not yield a significant habituation in the intensity of the startle response.

It is believed that the modulation of the startle response in the present study was driven mainly by infants' affective state during each of the three experimental conditions, rather than by differential levels of attention allocation as was presumably the case in Study 1. This contention is based on several levels of analysis. First, behavioral data gathered during infants' involvement in the peek-a-boo, bingo wheel, and arm-restraint episodes indicated that these three foreground activities were indeed successful in eliciting corresponding affective states in infants. That is, relative to infants' facial expression during the affectively neutral bingo wheel episode, infants demonstrated more positive affect during the peek-a-boo game, and more negative affect during the arm-restraint episode. In addition, the results indicated that the peek-a-boo game and the arm-restraint episode attracted infants' attention to a similar degree. This behavioral evidence that infants expressed different emotions during each foreground activity, yet they did not allocate differential levels of attention, suggests that infants' affective state rather than their attention level was responsible for the differences in startle reactions between the experimental conditions.

Interestingly, infants in the present study exhibited increased HP (i.e., decreased HR) during the pleasant and the neutral activities compared to the unpleasant activity. Given the relation established in the literature between decreased HR and increased attention (Graham et al., 1983; Lacey & Lacey, 1974; Porges, 1992), it is possible that infants were more attentive to the peek-a-boo game and the bingo wheel than to the arm-restraint episode. However, this explanation contradicts the behavioral data according to which infants allocated similar levels of attention to the peek-a-boo game and the arm-restraint episode. Alternative explanations for the pattern of autonomic reactivity obtained in this study are that the increase in HR during the arm-restraint episode relative to the bingo wheel and the peek-a-boo activities was a function of either overall greater emotional arousal or increased motor tension during arm restraint. The former explanation, once again, supports the motivational account for emotional modulation of startle. Clearly, more developmental research on emotional modulation of startle is required in order to tease apart affective and attentional processes and to substantiate the claim that emotional modulation of startle is present in infancy. The importance of the current research project and the potential applications of future studies on emotion-modulated startle in infancy are discussed in the next chapter.

SUMMARY AND GENERAL DISCUSSION

This research project was aimed at exploring whether the emotional modulation of startle is functional early in development, and to elucidate the specific characteristics of infants' startle response in an emotion-modulated startle paradigm. Two studies were performed to address these questions. In Study 1, acoustic startle probes were presented while infants were viewing photos of adult women posing happy, neutral, and angry facial expressions. The results of this study did not suggest that an emotion-modulated startle effect was present with this stimulus set. In contrast to the motivational priming hypothesis, infants' startle responses were significantly larger during the viewing of happy faces and significantly smaller during the viewing of angry faces, relative to their startle responses when affectively neutral expressions were presented. It was postulated that this effect was driven by allocation of more visual attention to angry faces and less visual attention to happy faces, rendering less attentional resources available for processing the auditory startle probe during the unpleasant versus the pleasant experimental condition. Infants' HR during the viewing of each type of facial expression as well as their differential looking time further supported this explanation.

In order to investigate whether emotion modulation of startle can be observed in 9-month-old infants, Study 2 replaced the pictorial foregrounds with more affectively salient activities. The pleasant, neutral, and unpleasant foreground activities were a game of peek-a-boo, a spinning bingo wheel, and an arm restraint episode, respectively. Infants' facial expressions during this study indicated that these activities were successful in eliciting the corresponding positive, neutral, and negative affect. There were no differences between the affective activities in the degree of infants' attention allocation. In accordance with the motivational priming account for emotion-modulated startle, infants demonstrated significant startle potentiation during the arm-restraint episode and startle inhibition during the peek-a-boo game, relative to the size of their startle response during the affectively-neutral spinning bingo wheel.

One important conclusion of the current research project is that emotional modulation of startle is present in infancy and can be manifested if infants are engaged in affectively arousing foreground tasks, such as those utilized in Study 2. If the affective content of the foreground tasks fails to elicit salient emotions in the infant, emotional modulation will not be exhibited and other psychological processes, such as attention, will influence the modulation of the startle response. In adults, the effect of the foreground stimuli on the participant is typically assessed via self-report measures, as in the evaluation of the IAPS (Greenwald et al., 1989; Lang et al., 1993). In infancy research, it is essential to incorporate behavioral measures to ensure that the desired affective state is induced, along with sufficiently elevated levels of arousal.

Importantly, even if affect is proven to be induced, there is still a need to control for alternative explanations for the modulation of the startle reflex. As discussed earlier, it is important to disentangle effects of attention from emotion on startle modulation. Even in the presence of salient and emotionally meaningful foreground signals, patterns of startle modulation might reflect indirect influences of affect on startle, mediated via modality-selective attention (Anthony & Graham, 1983). In adults, this potential confound has been rigorously studied and it is now established that startle modulation by emotion exists regardless of the modality of the reflex-eliciting probes (Bradley et al., 1990). Similar studies are necessary in infants before emotional and attentional explanations can be further teased apart.

Another potential confound that has to be carefully considered is motor influences on blink responsivity. Vecchierini-Blineau and Guiheneue (1984), in noting that the EMG components of infants' blink reflexes elicited by electrical stimulation on the forehead were facilitated during crying, proposed that facial muscle activity as well as arousal in brain stem reticular pathways contributed to the reflex augmentation. Thus, a potential motor explanation for the present results is that the changes in blink size were secondary to affect-induced changes in facial musculature. In the present research, the facial coding system utilized was relatively unrefined, mainly due to methodological issues concerning coding of infant facial expression (Oster, Hegley, & Nagel, 1992). However, baseline activity of the *orbicularis oculi* muscle was measured under the assumption that this muscle has the greatest contribution to the eyeblink startle response. Indeed, Study 1 revealed no differences between all conditions in the activity of this muscle prior to blink onset, whereas Study 2 showed differences between the two affective conditions (pleasant or unpleasant) relative to the neutral condition, and no significant differences between the pleasant and the unpleasant conditions. Nonetheless, it is possible that other facial muscles contributed to infants' potentiated startle response. This potential confound has not been addressed in research of startle modulation in infants, nor has it been fully studied in adults. Future research might incorporate a wider recording of facial EMG activity and/or a more refined facial coding system to shed more light on the mediating role of facial motor activity on the emotional modulation of startle.

The presence of the emotion-modulated startle effect in infancy has intriguing implications for the understanding of infants' emotionality. First, the results obtained here provide additional support to the idea that from a very early age, the emotional world of humans is organized around the appetitive and aversive motive systems (Davidson & Fox, 1988). These systems determine the organism's behavioral and physiological response to affect-eliciting cues. In the present research project, this was manifested through the differential patterns of facial expressions and autonomic reactivity that were exhibited by the infant in response to the specific foreground stimuli. These reactions were presumably a function of the mediating effect of the correspondingly activated motivational system. Moreover, this research demonstrated that information in the environment that is linked to the engaged motivational system is more accessible and has greater output strength than other information. Thus, infants' startle responses to acoustic startle probes were potentiated when the defensive motive system was engaged, whereas during the activation of the appetitive motive system, infants' startle response was inhibited. The modulatory effect of infants' emotional organization on other processing operations in the brain, such as memory associations, representations, and action programs should be further explored in future research. Also, future research is required in order to follow up the null findings obtained here with respect to the influence of the affective valence of the foreground stimuli on the latency of the startle response. It may be that this deviation from the typical adult pattern of startle modulation signals a difference between infants and adults in the mechanisms contributing to emotional modulation of startle.

Above and beyond the contribution of the current findings to the understanding of infants' emotional organization, the presence of the emotion-modulated effect on startle responses in infancy has important implications for research on infants' perception of affective stimuli. Given the paucity of means by which infants' affective experiences can be explored, our understanding of infants' responses to stimuli designed to elicit emotion is incomplete. Thus, startle paradigms might be exceptionally valuable tools in investigating infants' processing of the affective qualities of a range of stimuli. For example, one of the conclusions drawn in Study 1 was that angry facial expressions might not have a salient affective meaning for 9-month-old infants, since these stimuli did not engage the defensive motive system (as would be seen through a modulation of the startle response) during the presentation of these stimuli. It is believed that affective manipulations of the eyeblink reflex could also prove to be an informative developmental measure of infants' emerging responsiveness to other emotional signals. Furthermore, given the inevitable link between the processing of affective stimuli and the individual's induced emotion, startle probe paradigms may provide a useful window into the organism's underlying affective state. This methodological advantage is invaluable in terms of adding to the scarce resources available to researchers interested in objective measures of infant emotion.

Another line of research that could benefit from the utilization of emotion-modulated startle paradigms is the study of individual differences in infants' emotionality. For instance, the eyeblink reflex might aid in examining the effect of specific emotional cues on infants with different temperamental dispositions (Schmidt & Fox, 1998a), or even assist in categorizing infants into different temperamental groups.

Schmidt and Fox (1998a) suggested that there might be a subset of infants who are biologically predisposed to a low threshold for arousal in forebrain limbic structures that regulate negative affect. These infants may be unable to successfully regulate negative affect, which might well be manifested in greater potentiation of the startle response in the context of unpleasant emotional signals. Moreover, since startle research has facilitated the understanding of emotional processes in psychopathological populations (see Grillon & Baas, 2003 for review), it might be possible to further utilize startle paradigms to increase the understanding of normal and abnormal emotional development in infancy and early childhood. For example, the observation that startle is affected by fear and anxiety, as well as other emotional states, suggests that abnormalities in startle modulation by emotionally salient stimuli may relate to disturbances in emotional and motivational states (Grillon & Baas, 2003).

From a procedural point of view, it is important to note that startle methodology can be applied to the study of emotional development with relative ease. The procedures involved in eliciting, recording, and quantifying startle are straightforward, and the required equipment in not overly expensive. Thus, startle research can be developed not only in advanced psychophysiological settings, but also in laboratories that specialize in the study of emotion. Moreover, the methods involved in emotion-modulated startle paradigms are non-invasive and typically impose few demand characteristics on the participant, rendering them relatively easy to use in developmental research.

Above and beyond the actual presence of startle modulation in infancy, the two studies described here revealed some developmentally intriguing characteristics of infants' startle response that warrant further discussion. First, in both studies, infants' latency to respond was considerably slower than the latency typically manifested in adults. While in adults, the startle response to an acoustic probe occurs 30-40 ms after probe onset, infants' peak EMG activity across all experimental conditions occurred at around 90 ms. Slow latencies were also reported with 5-month-old infants in a similar emotion-modulated startle paradigm (Balaban, 1995), and with 4-month-old infants in a modality-selective startle paradigm (Anthony & Graham, 1983). Although the startle reflex is present at birth, and the neural circuit underlying the eyeblink startle response to an acoustic probe involves just a few synapses (Davis et al., 1999), a possible explanation for the slower latencies observed in infants might be related to the maturation of the startle pathway in infants. In particular, it is possible that the myelination of the neurons that make up the startle circuit (i.e., cochlear root neurons, neurons in the PnC, and motoneurons in the spinal cord) is not fully developed in infancy, attenuating the speed of the startle reflex.

Another interesting characteristic of infants' startle response found in the current research is the relatively low response probability (i.e., the relatively frequent occurrence of undetectable startle responses). In Study 1, the probability of eliciting a non-zero blink response averaged 58%, whereas in Study 2, mean response probability across the experimental conditions was 75%. Similar frequencies of response were also reported in 5-month-old infants (Balaban, 1995). Since response probabilities are not commonly reported in research on the emotional modulation of startle, more studies are required to investigate the developmental course of startle responsivity. Moreover, it would be interesting to explore the influence of external (e.g., characteristics of the foreground stimulus and the startle probe) and internal (e.g., attentional) factors on response probability among infants, children, and adults. The relatively frequent occurrence of no detectable startle response to the auditory probes further calls for the reporting of startle amplitude in startle research with infants. This measure averages peak EMG for trials with non-zero responses only, whereas the more commonly reported startle magnitude averages peak EMG for all non-rejected trials regardless of whether startle responses are detectable. Given that startle magnitude, by definition, is influenced by both amplitude and response probability, this measure might not be a valid index of reflex size when the frequency of response is relatively low. On the other hand, measures of amplitude possess the disadvantage of requiring a subjective decision regarding the presence of a startle reaction in each experimental trial. It is therefore advisable to include both magnitude and amplitude measures, or if only startle magnitude is considered, to supplement this information with reports of response probability.

In summary, this research project suggests the presence of the emotion-modulated startle effect in 9-month-old infants in the context of emotionally arousing and meaningful stimulation. Nonetheless, it also raises the need for future research to follow up the results obtained here in order to fully tease apart the contribution of various factors, such as attention and activity of other facial muscles to startle modulation in infancy. Additional research is also required in order to extend the knowledge about the characteristics of startle reactivity in infancy. By further addressing these issues, the eyeblink startle reflex, in conjunction with other behavioral and physiological measures, can be used to probe infants' emotional state and their processing of and responsiveness to various emotional stimuli, as well as individual differences in emotional processes.

TABLES

Table 1

Mean Number of Trials (and Standard Deviations) Accepted for the Analyses of Peak Magnitude and Peak Amplitude of Infants' Startle Responses, and Response Probabilities during the Viewing of Happy, Neutral, and Angry Facial Expressions.

	Mean number of trials overall (SD)	Mean number of trials during happy faces (SD)	Mean number of trials during neutral faces (SD)	Mean number of trials during angry faces (SD)
Peak magnitude	17.57 (7.22)	5.83 (2.48)	6.03 (2.41)	5.70 (2.73)
Peak amplitude	10.07 (7.13)	3.43 (2.47)	3.43 (2.39)	3.20 (2.75)
Response probability	.58 (.31)	.59 (.32)	.57 (.31)	.58 (.38)

Table 2

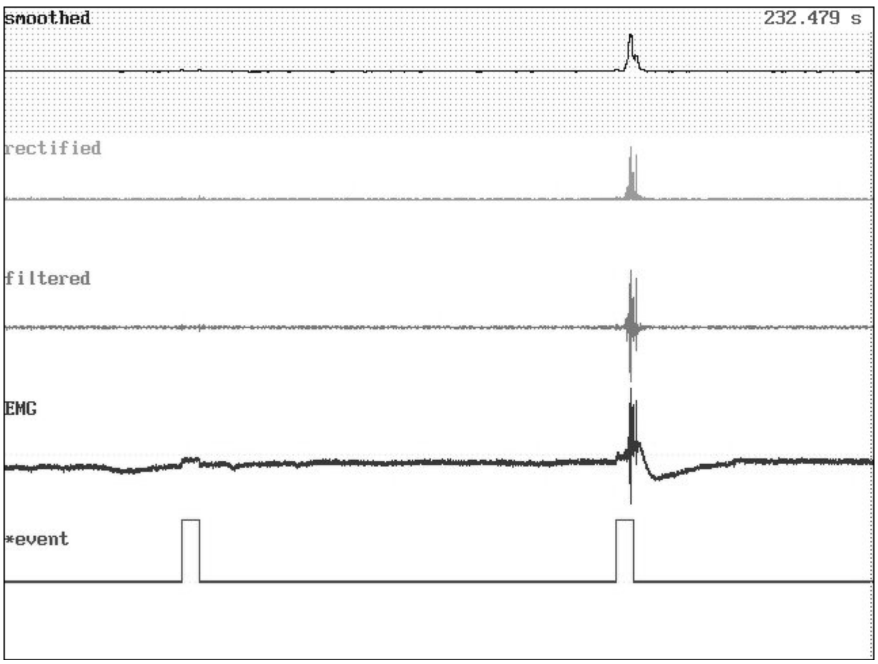
Mean Number of Trials (and Standard Deviations) Accepted for the Analyses of Peak Magnitude and Peak Amplitude of Infants' Startle Responses, and Response Probabilities during Peek-A-Boo, Bingo, and Arm Restraint Episodes.

	Mean number of trials overall (SD)	Mean number of trials during peek-a-boo (SD)	Mean number of trials during bingo wheel (SD)	Mean number of trials during arm restraint (SD)
Peak magnitude	15.61 (2.27)	5.00 (1.17)	5.70 (.56)	4.91 (1.38)
Peak amplitude	11.78 (3.58)	2.91 (1.56)	5.17 (.98)	3.70 (1.85)
Response probability	.75 (.20)	.61 (.30)	.90 (.13)	.72 (.31)

FIGURES

Figure 1

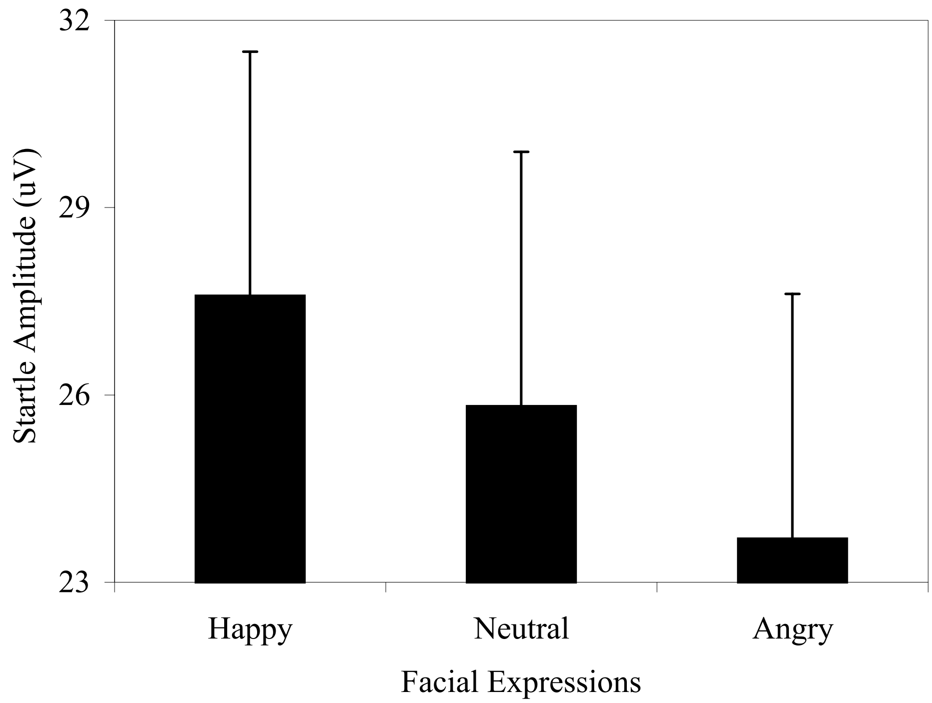
An Example of the Raw, Filtered, Rectified, and Smoothed EMG Signal.



Note. This image contains 7 s of EMG data. The first event mark in the event mark channel represents the onset of picture presentation, whereas the second event marks the onset of the startle probe. Each dotted line in the smoothes signal represents an increment of 5 μV.

Figure 2

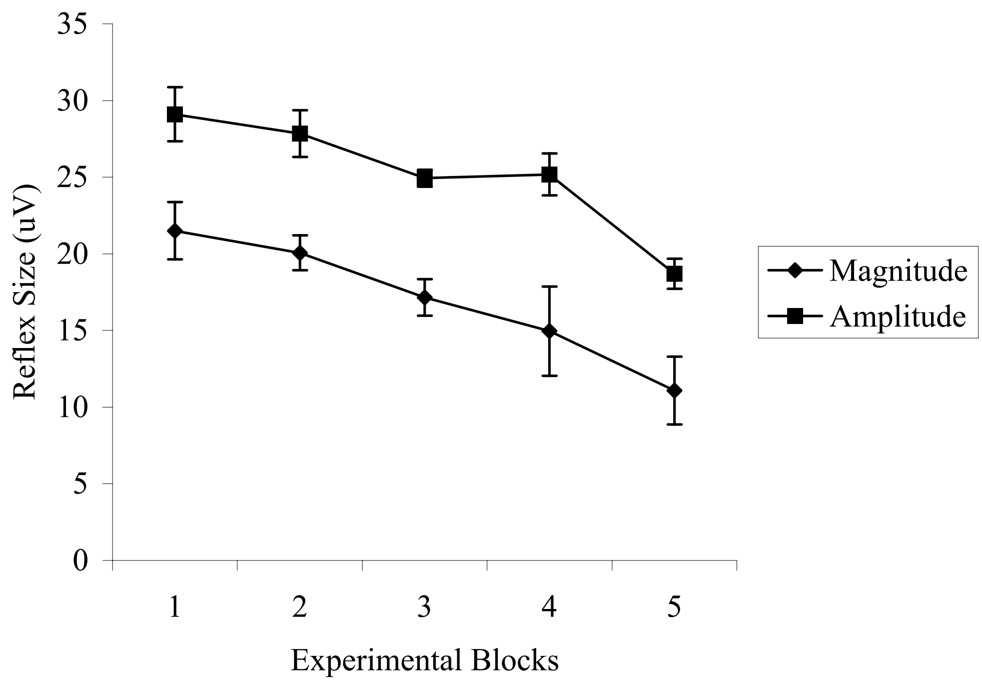
Peak Amplitude of Infants' Startle Responses Elicited during the Viewing of Happy, Neutral, and Angry Facial Expressions.



Note. Error bars indicate 1 SE.

Figure 3

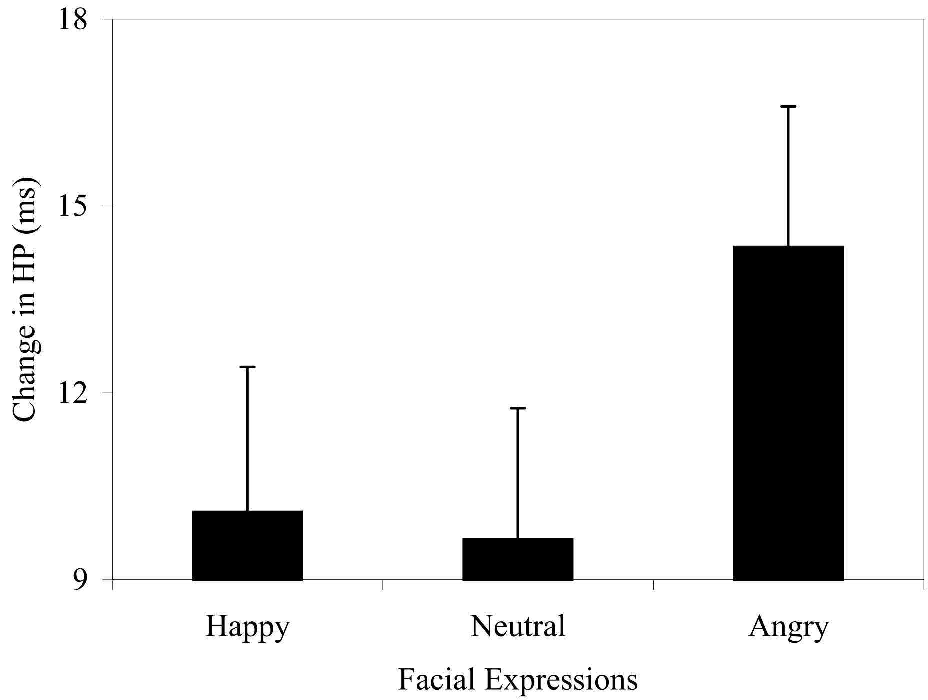
<u>Habituation in Peak Magnitude and Peak Amplitude of Infants' Startle Responses during Picture Viewing Across Experimental Blocks.</u>



Note. Error bars indicate 1 SE.

Figure 4

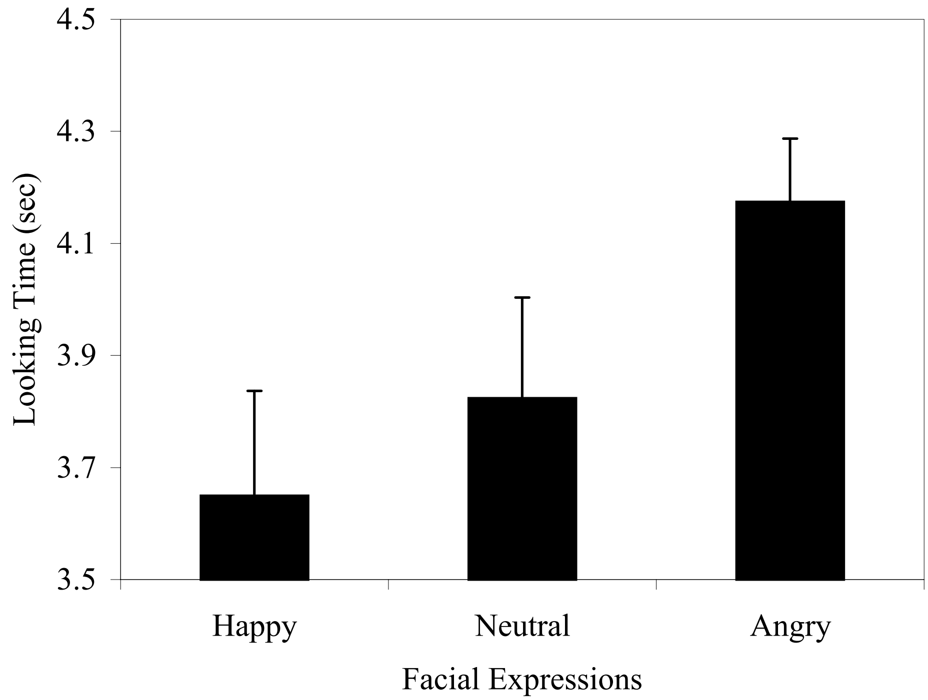
Magnitude of Change in Infants' Heart Period following the Presentation of Happy, Neutral, and Angry Facial Expressions.



Note. Error bars indicate 1 SE.

Figure 5

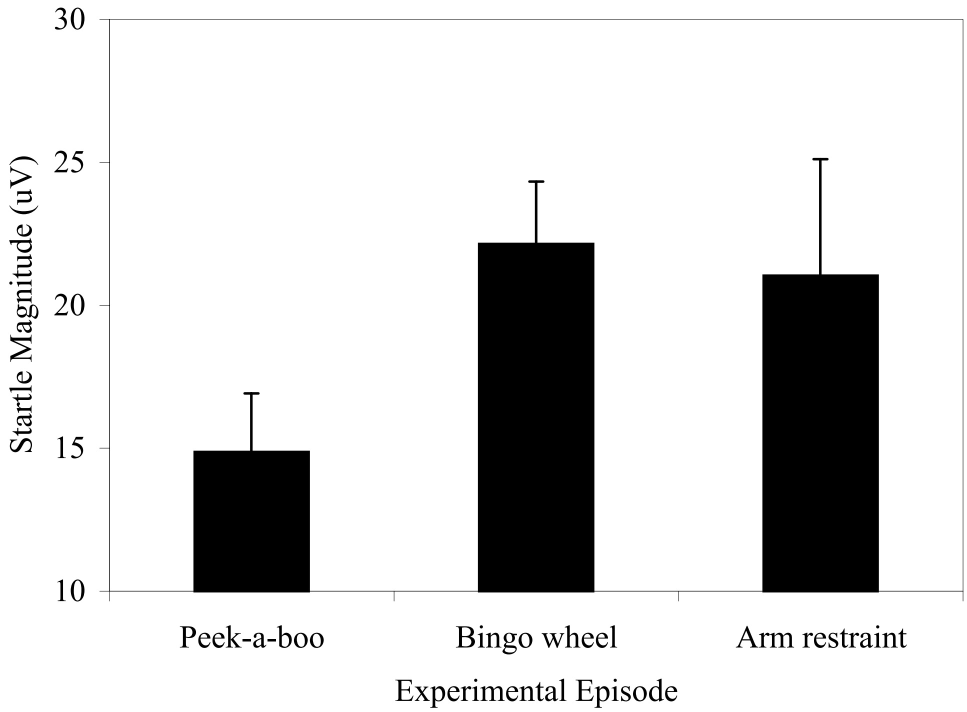
Infant's Duration of Looking to Pictures of Happy, Neutral, and Angry Facial Expressions.



Note. Error bars indicate 1 SE.

Figure 6

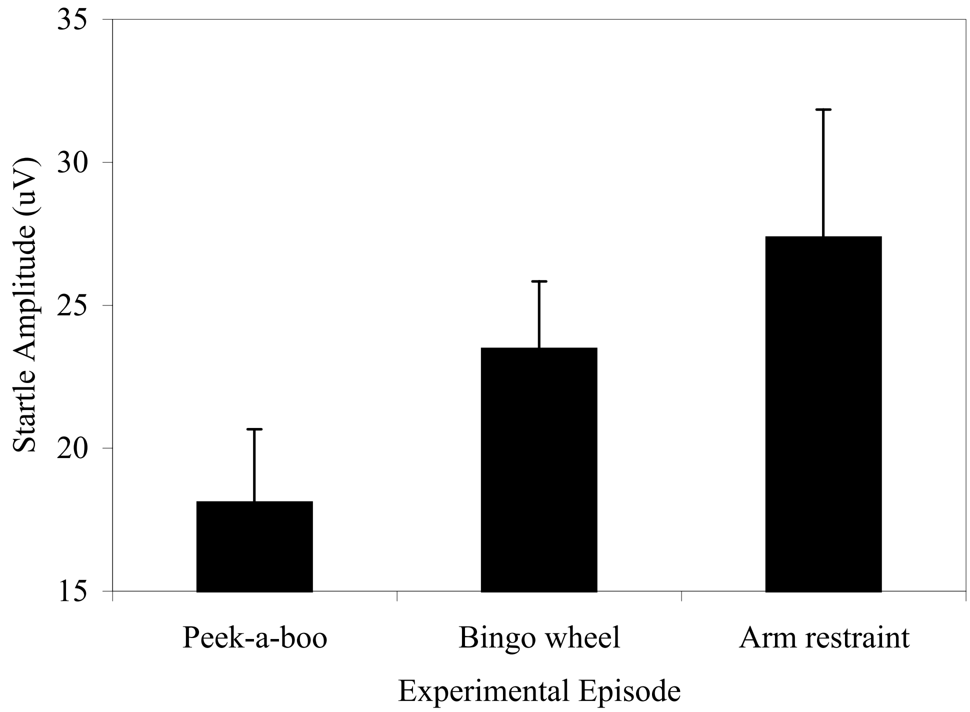
Peak Magnitude of Infants' Startle Responses Elicited during Peek-A-Boo, Bingo, and Arm Restraint Episodes.



Note. Error bars indicate 1 SE.

Figure 7

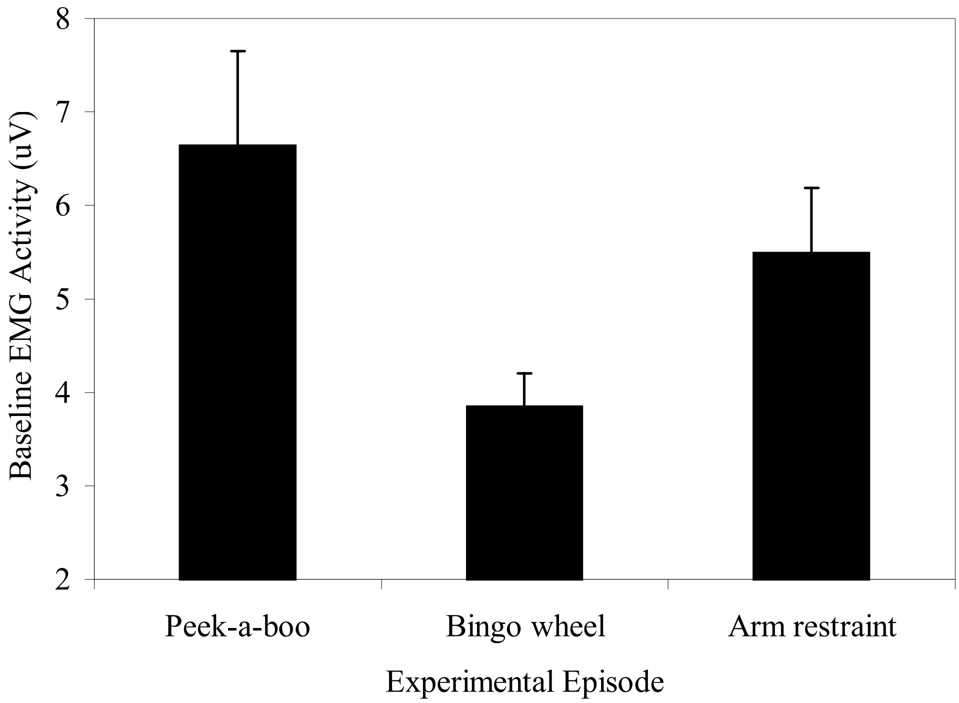
Peak Amplitude of Infants' Startle Responses Elicited during Peek-A-Boo, Bingo, and Arm Restraint Episodes.



Note. Error bars indicate 1 SE.

Figure 8

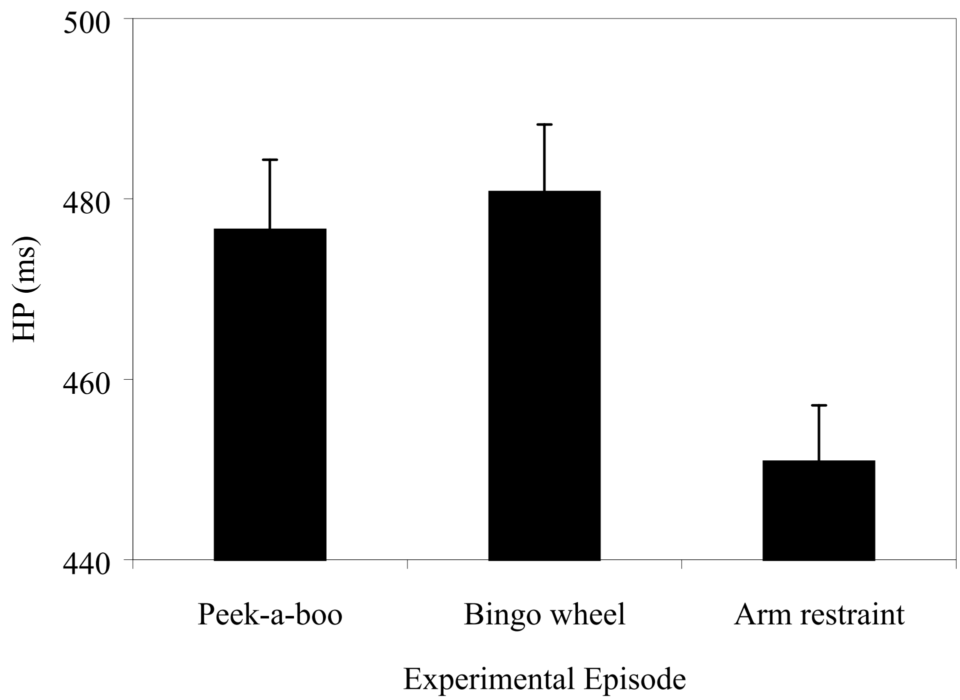
Baseline EMG Activity during Peek-A-Boo, Bingo, and Arm Restraint Episodes.



Note. Error bars indicate 1 SE.

Figure 9

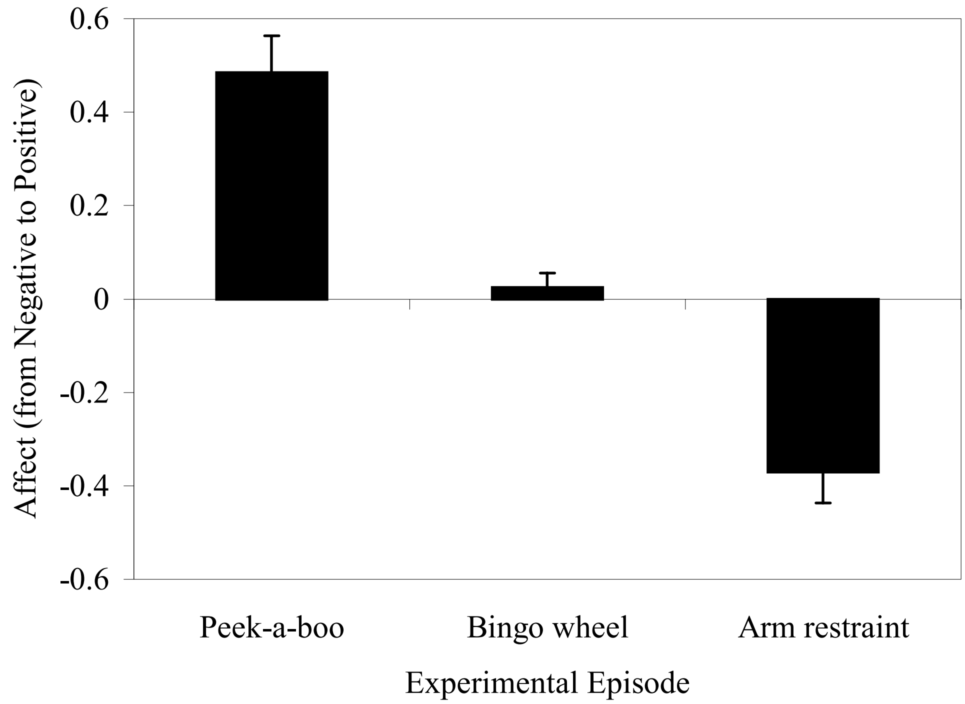
Infants' Heart Period during Peek-A-Boo, Bingo, and Arm Restraint Episodes.



Note. Error bars indicate 1 SE.

Figure 10

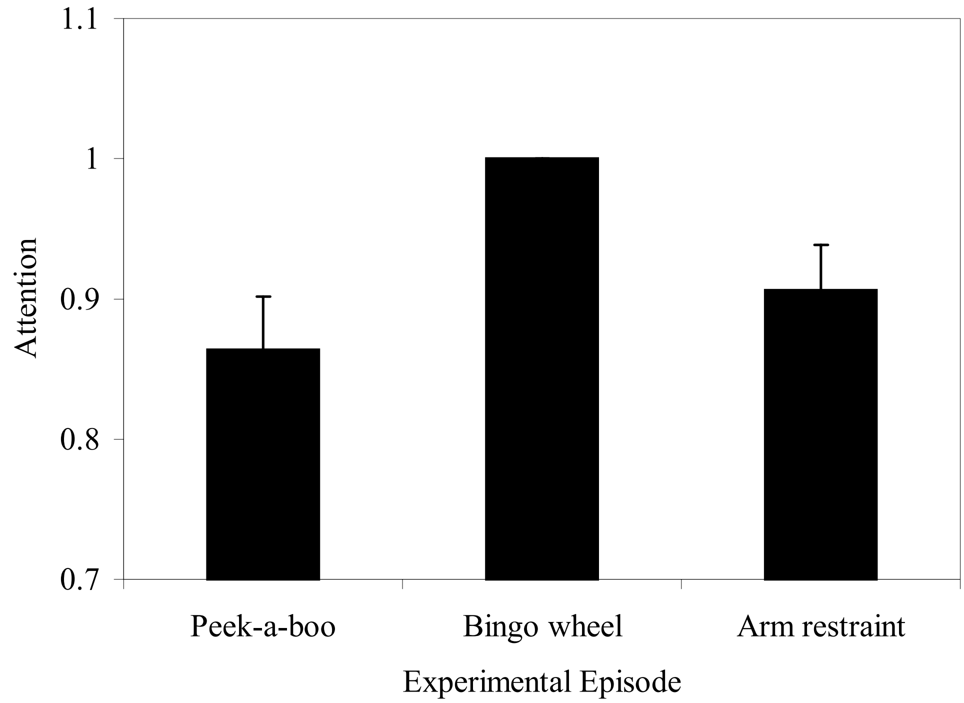
Infant's Affect during Peek-A-Boo, Bingo, and Arm Restraint Episodes.



Note. Error bars indicate 1 SE.

Figure 11

<u>Infant's Attention during Peek-A-Boo, Bingo, and Arm Restraint Episodes.</u>



Note. Error bars indicate 1 SE.

APPENDICIES

Appendix A

Recruitment Letter

Dear Parents,

We are writing from the Child Development Laboratory at the University of Maryland to tell you about an exciting study that we are conducting. For the past fifteen years, we have been studying the ways in which children develop, socially and emotionally, from infancy throughout childhood. Our research has been recognized on television programs such as Dateline, 20/20, and Good Morning America, as well as in the written media: Life and USA Today.

We are currently recruiting families with 9-month-old infants to participate in a study investigating infants' emotional development. Your name, home address, and your child's date of birth were given to us from commercially available mailing lists, and we hope very much to have you among our participants. The study will focus on infants' behavioral and physiological reactivity in response to emotional stimuli. Funding for this project is being provided by the National Institute of Child Health and Human Development (National Institutes of Health).

Upon receiving the completed questionnaire (enclosed), we may contact you by phone to provide you with greater details and to invite you to participate in the study. To thank you for your participation, you will receive $10 and your child will receive a small toy. Please note that returning the enclosed questionnaire does not commit you to our project in any way and all information provided will be kept private and confidential.

If you have any questions, please feel free to contact us at (301) 405-8315. Our research would be impossible without the invaluable assistance provided by the families that participate in our studies. We appreciate your time, interest, and any information you can provide us with. Thank you very much.

Sincerely,

Nathan Fox, Ph.D.	Dalit Marshall
Professor	Doctoral Candidate
Department of Human Development	Department of Human Development

Appendix B

Child Survey

University of Maryland – The Child Development Lab

Child's birth date: _____________

Child's gender: Female ____ Male ____

Child's full name: ____

Child's sibling order: Child is ____ of ____ (ex. 1st of 3)

Was your child born within 2 weeks of her/his due date? Yes ____ No ____

What was your child's method of delivery? Natural ___ Cesarean Section ___ Other ___
If "other", please explain: ____

Did you and/or your child experience any birth complications? Yes ____ No ____
If "yes", please explain: ____

How many days did your child spend in the hospital after birth? ____________________

Has your child experienced any serious illnesses or problems in development since birth?
Yes ____ No ____
If "yes", please explain: ____

Has your child received long-term medication? Yes ____ No ____
If "yes" please explain: ____

Ethnic Group:	Mother:	Father:
	____ African-American	____ African-American
	____ Asian	____ Asian
	____ Caucasian	____ Caucasian
	____ Hispanic	____ Hispanic
	____ Other	____ Other

Handedness: Mother: Left ____ Right ____ Father: Left ____ Right ____

May we contact you about our research project? Yes ____ No ____
Mother's name: ____
Address: ____
____
Phone: H ( ) ______________________ W ( ) ______________________

Appendix C

Informed Consent Form for Study 1

Title of project:	**Emotional modulation of startle in infants**. Funding for this research is being provided by the National Institutes of Health (HD #17899).
Statement of consent:	I state that I am over 18 years of age, in good physical health, and wish to participate with my child in a program of research being conducted by Dr. Nathan Fox in the Department of Human Development, University of Maryland, College Park, MD 20742.
Purpose:	The purpose of this research is to examine infants' physiological reactions in response to pictures with emotional content.
Procedures:	My baby will sit on my lap and an experimenter will place two small electrodes underneath her/his right eye in order to collect eyeblink startle responses. Two additional electrodes will be placed on my baby's chest to record heart rate. Following the placement of the electrodes, I will be given earplugs and a sun visor so that I can see my baby but not see the computer screen. My baby will watch some pictures on a computer screen. The pictures will include a photograph of a woman smiling, a photograph of a woman with an angry facial expression, and a photograph of a woman with a neutral facial expression. During or following stimulus presentation, short loud noises will be presented from the two speakers located on the wall. The acoustic probe is designed to elicit an eyeblink startle response in my baby. Each acoustic probe will have a duration of 50 ms, and a sound pressure level of 100 dB. I understand that several safety mechanisms are used to ensure that the sound level does not exceed 100 dB. First, a sound pressure level meter is used to calibrate the sound level at the infant's ear. Second, the intensity and the duration of the acoustic probe were defined in software, and consequently, when triggered, only an acoustic probe of these exact properties can be presented. This procedure will last approximately 15 minutes. At the end of this procedure, I will be asked to complete a questionnaire on my baby's temperament. I understand that I will be paid $10.00 for my visit at the lab and that my child will be given a small toy as compensation for participation.
Confidentiality:	I understand that all information collected during the course of the analysis will remain confidential and that my name or my child's name will not be identified at any time, except to the extent required by law, which includes the requirement to report child abuse or neglect. The data will be grouped with data others provide for reporting and presentation.
Risks:	I understand that the procedures described above are widely used in child development research and are not physically harmful to my child or me. I understand that some infants may become upset during the picture presentation and/or during the presentation of the acoustic probes. I understand that if at any time I feel that I am not comfortable with the experimental procedure or that my baby is too distressed, I may terminate the procedure. I understand that there are no known long-term effects associated with the tasks or events experienced during these visits.

Benefits, Freedom to withdraw and to ask questions:	I understand that the experiment is not designed to help my child or me personally, but that the investigator hopes to learn more about infants' reactivity to emotional stimuli. I have been given the opportunity to ask questions about this study, and have been answered to my satisfaction. If I should have any further questions, I understand that I am free to ask them at any time during the procedure. I understand that I am free to refuse to participate in any part of the study I choose, and that I may withdraw at any time without penalty.
Medical care:	I understand that the University of Maryland does not provide any medical or hospitalization insurance coverage for participants in the research study, nor will the University of Maryland provide any compensation for any injury sustained as a result of participation in the research study except as required by law.
Principal Investigator:	Nathan Fox Dept. of Human Development 3304 Benjamin Bldg. University of Maryland College Park, MD 20742-1131 (301) 405-2816

Printed name of parent: ________________________________

Signature: ________________________________

Date: ________________________________

Child's name: ________________________________

Child's date of birth: ________________________________

Appendix D

Face Stimulus Set used in Study 1

Appendix E

Informed Consent Form for Study 2

Title of project:	**Emotional modulation of startle in infants**. Funding for this research is being provided by the National Institutes of Health (HD #17899).
Statement of consent:	I state that I am over 18 years of age, in good physical health, and wish to participate with my child in a program of research being conducted by Dr. Nathan Fox in the Department of Human Development, University of Maryland, College Park, MD 20742.
Purpose:	The purpose of this research is to examine infants' physiological reactions while engaged in emotion-eliciting activities.
Procedures:	My baby will sit on my lap and an experimenter will place two small electrodes underneath her/his right eye in order to collect eyeblink startle responses. Two additional electrodes will be placed on my baby's chest to record heart rate. Following the placement of the electrodes, I will be given earplugs to minimize the impact I may have on my baby's responses. My baby will participate in 3 activities presented in a random order. One of the activities is a peek-a-boo game with the experimenter. A second activity is a play session with a toy during which I will sometimes be asked to restrain his/her arms. The third activity involves watching a spinning bingo wheel with a varying number of balls. During these activities, short loud noises will be presented from the two speakers on the wall. The acoustic probe is designed to elicit an eyeblink startle response in my baby. Each acoustic probe will have a duration of 50 ms, and a sound pressure level of 100 dB. I understand that several safety mechanisms are used to ensure that the sound level does not exceed 100 dB. First, a sound pressure level meter is used to calibrate the sound level at the infant's ear. Second, the intensity and the duration of the acoustic probe were defined in software, and consequently, when triggered, only an acoustic probe of these exact properties can be presented. This procedure will last approximately 15 minutes. The procedure will be videotaped in order to later be able to code my baby's emotional expressions during each of the experimental activities. At the end of this procedure, I will be asked to complete a questionnaire on my baby's temperament. I understand that I will be paid $10.00 for my visit at the lab and that my child will be given a small toy as compensation for participation.
Confidentiality:	I understand that my baby will be assigned an identification number and will not be identified by name on any form of data, including videotapes. All information collected during this study will remain confidential, except to the extent required by law, which includes the requirement to report child abuse or neglect. All the collected data will be stored at the Child Development Laboratory, which is alarmed after business hours and to which only the laboratory staff has access.
Risks:	I understand that the procedures described above are widely used in child development research and are not physically harmful to my child or me. I understand that some infants may become upset during the different activities and/or during the presentation of the acoustic probes. I understand that if at

any time I feel that I am not comfortable with the experimental procedure or that my baby is too distressed, I may terminate the procedure. I understand that there are no known long-term effects associated with the tasks or events experienced during this visit.

Benefits, Freedom to withdraw and to ask questions:	I understand that the experiment is not designed to help my child or me personally, but that the investigator hopes to learn more about infants' reactivity to emotional stimuli. I have been given the opportunity to ask questions about this study, and have been answered to my satisfaction. If I should have any further questions, I understand that I am free to ask them at any time during the procedure. I understand that I am free to refuse to participate in any part of the study I choose, and that I may withdraw at any time without penalty.
Medical care:	I understand that the University of Maryland does not provide any medical or hospitalization insurance coverage for participants in the research study, nor will the University of Maryland provide any compensation for any injury sustained as a result of participation in the research study except as required by law.
Principal Investigator:	Nathan Fox Dept. of Human Development 3304 Benjamin Bldg. University of Maryland College Park, MD 20742-1131 (301) 405-2816

Printed name of parent: ________________________________

Signature: ________________________________

Date: ________________________________

Child's name: ________________________________

Child's date of birth: ________________________________

REFERENCES

Aggleton, J. P., & Mishkin, M. (1986). The amygdala: Sensory gathway to the emotions. In R. Plutchik & H. Kellerman (Eds.), *Emotion: Theory, research and experience* (Vol. 3). New York: Academic Press.

Anthony, B. J., & Graham, F. K. (1983). Evidence for sensory-selective set in young infants. *Science, 220,* 742-744.

Anthony, B. J., & Graham, F. K. (1985). Blink reflex modification by selective attention: Evidence for the modulation of "automatic" processing. *Biological Psychology, 21,* 43-59.

Anthony, B. J., & Putnam, L. E. (1985). Cardiac and blink concomitants of attentional selectivity: A comparison of adults and young children. *Psychophysiology, 22,* 508-516.

Anthony, B. J., Zeigler, B. L., & Graham, F. K. (1987). Stimulus duration as an age-dependent factor in reflex blinking. *Developmental Psychobiology, 20,* 285-297.

Balaban, M. T. (1995). Affective influences on startle in five-month-old infants: Reactions to facial expressions of emotion. *Child Development, 66,* 28-36.

Balaban, M. T., Anthony, B. J., & Graham, F. K. (1985). Modality-repetition and attentional effects on reflex blinking in infants and adults. *Infant Behavior and Development, 8,* 443-457.

Balaban, M. T., Anthony, B. J., & Graham, F. K. (1989). Prestimulation effects on blink and cardiac reflexes of 15-month human infants. *Developmental Psychobiology, 22,* 115-127.

Barrera, M. E., & Maurer, D. (1981). Discrimination of strangers by the three-month-old. *Child Development, 52*, 559-563.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269,* 1115-1118.

Berg, W. K., & Balaban, M. T. (1999). Startle elicitation: Stimulus parameters, recording techniques, and quantification. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (pp. 6-18). New York, NY: Cambridge University Press.

Blumenthal, T. D. (1999). Short lead interval startle modification. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (pp. 51-71). New York, NY: Cambridge University Press.

Bohlin, G., & Graham, F. K. (1977). Cardiac deceleration and reflex blink facilitation. *Psychophysiology, 14,* 423-430.

Bradley, M. M., Codispoti, M., Cuthbert, B. N., & Lang, P. J. (2001). Emotion and motivation I: Defensive and appetitive reactions in picture processing. *Emotion, 1*, 276-298.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1990). Startle reflex modification: Emotion or attention? *Psychophysiology, 27,* 513-522.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1991). Startle and emotion: Lateral acoustic probes and the bilateral blink. *Psychophysiology, 28,* 285-295.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1993). Pictures as prepulses: Attention and emotion in startle modification. *Psychophysiology, 30,* 541-545.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1996). Lateralized startle probes in the study of emotion. *Psychophysiology, 33,* 156-161.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1999). Affect and the startle reflex. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (pp. 157-183). New York, NY: Cambridge University Press.

Bradley, M. M., Gianaros, P., & Lang, P. J. (1995). As time goes by: Stability of affective startle modulation [Abstract]. *Psychophysiology, 32* (Suppl. 1), S21.

Bradley, M. M., & Lang, P. J. (2000). Measuring emotion: Behavior, feeling, and physiology. In R. Lane & L. Nadel (Eds.), *Cognitive neuroscience of emotion* (pp. 242-276). New York: Oxford University Press.

Bradley, M. M., Lang, P. J., & Cuthbert, B. N. (1993). Emotion, novelty, and the startle reflex: Habituation in humans. *Behavioral Neuroscience, 107,* 970-980.

Bradley, M. M., Zack, J., & Lang, P. J. (1994). Cries, screams, and shouts of joy: Affective responses to environmental sounds [Abstract]. *Psychophysiology, 31* (Suppl. 1), S29.

Braff, D. L., & Geyer, M. A. (1990). Sensorimotor gating and schizophrenia: Human and animal model studies. *Archives of General Psychiatry, 47,* 181-188.

Bronson, G. W. (1972). Infants' reactions to unfamiliar persons and novel objects.*Monographs of the Society for Research in Child Development*, 37(3, Serial No. 148).

Brown, J. S., Kalish, H. I., & Farber, I. E. (1951). Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. *Journal of Experimental Psychology, 41,* 317-328.

Bruner, J. S., & Sherwood, V. (1976). Early rule structure: The case of "peekaboo". In R. Harre (ed.), *Life Sentences* (p. 55-62). London: John Wiley.

Byrne, E. A., & Porges, S. W. (1993). Data-dependent filter characteristics of peak-valley respiratory sinus arrhythmia estimation: A cautionary note. *Psychophysiology, 30,* 397-404.

Cahill, L., & McGaugh, J. L. (1990). Amygdaloid complex lesions differentially affect retention of tasks using appetitive and aversive reinforcement. *Behavioral Neuroscience,* 104, 532-543.

Calkins, S. D., Fox, N. A., & Marshall, T. R. (1996). Behavioral and physiological antecedents of inhibited and uninhibited behavior. *Child Development, 67,* 523-540.

Campeau, S., & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *Journal of Neuroscience, 15,* 2301-2311.

Caron, R. F., Caron, A. J., & Myers, R. S. (1985). Do infants see emotional expressions in static faces? *Child Development, 56,* 1552-1560.

Codispoti, M., Bradley, M. M., & Lang, P. J. (1996). Probing the mind's eye: Reflex modulation for briefly presented pictures [Abstract]. *Psychophysiology, 33* (Suppl. 1), S29.

Cook, E. W., Davis, T. L., Hawk, L. W., Spence, E. L., & Gautier, C. H. (1992). Fearfulness and startle potentiation during aversive stimuli. *Psychophysiology, 29,* 633-645.

Cook, E. W., III, Hawk, L. W., Davis, T. L., & Stevenson, V. E. (1991). Affective individual differences and startle reflex modulation. *Journal of Abnormal Psychology, 100,* 5-13.

Cook, E. W., III, Hawk, L. W., Hawk, T. M., & Hummer, K. (1995). Affective modulation of startle in children [Abstract]. *Psychophysiology, 32* (Suppl. 1), S31.

Cuthbert, B. N., Bradley, M. M., & Lang, P. J. (1996). Probing picture perception: Activation and emotion. *Psychophysiology, 33,* 103-111.

Davidson, R. J., & Fox, N. A. (1988). Cerebral asymmetry and emotion: Developmental and individual differences. In D. L. Molfese & S. J. Segalowitz (Eds.), *Brain lateralization in children: Developmental implications* (pp. 191-206). New York: Guilford.

Davis, M. (1984). The mammalian startle response. In R. C. Eaton (Ed.), *Neural mechanisms of startle behavior* (pp. 287-351). New York: Plenum Press.

Davis, M., & Astrachan, D. I. (1978). Conditioned fear and startle magnitude: Effects of different footshock or backshock intensities used in training. *Journal of Experimental Psychology: Animal Behavior Processes, 4,* 95-103.

Davis, M., Walker, D. L., & Lee, Y. (1999). Neurophysiology and neuropharmacology of startle and its affective modulation. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience,* *cognitive science, and clinical science* (pp. 95-113). New York, NY: Cambridge University Press.

Dawson, M. E., Schell, A. M., & Böhmelt, A. H. (1999). Startle modification: Introduction and overview. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (pp. 6-18). New York, NY: Cambridge University Press.

de Haan, M., & Nelson, C. A. (1999). Brain activity differentiates face and object processing by 6-month-old infants. *Developmental Psychology, 34*, 1114-1121.

Dichter, G. S., Tomarken, A. J., & Baucom, B. R. (2002). Startle modulation before, during and after exposure to emotional stimuli. *International Journal of Psychophysiology, 43*, 191-196.

Dickinson, A., & Dearing, M. F. (1979). Appetitive-aversive interactions and inhibitory processes. In A. Dickinson & R. A. Boakes (Eds.), *Mechanisms of learning and motivation* (pp. 203-231). Hillsdale, NJ: Erlbaum.

Emde, R. N., & Harmon, R. J. (1972). Endogenous and exogenous smiling systems in early infancy. *Journal of the American Academy of Child Psychiatry*, *11*, 177-200.

Erickson, L. M., Levenston, G. K., Curtin, J., Goff, A., & Patrock, C. J. (1995). Affect and attention in startle modulation: Picture perception and anticipation [Abstract]. *Psychophysiology, 32* (Suppl. 1), S30.

Erlichman, H., Brown, S., Zhu, J., Warrenburg, S. (1995). Startle reflex modulation during exposure to pleasant and unpleasant odors. *Psychophysiology, 32,* 150-154.

Everitt, B. J., & Robbins, T. W. (1992). Amygdala-ventral striated interactions and reward related processes. In J. Aggelton (Ed.), *The amygdala*. New York: Wiley.

Fernald, A. (1993). Approval and disapproval: Infant responsiveness to vocal affect in familiar and unfamiliar languages. *Child Development, 64,* 657-674.

Field, T. M. (1982). Individual differences in the expressivity of neonates and young infants. In R. Feldman (Ed.), *Development of nonverbal behavior in children*. New York: Springer Verlag.

Funayama, E. S., Grillon, C., Davis, M., & Phelps, E. A. (2001). A double dissociation in the affective modulation of startle in humans: Effects of unilateral temporal lobectomy. *Journal of Cognitive Neuroscience, 13,* 721-729.

Geisser, S., & Greenhouse, S. W. (1958). An extension of Box's results on the use of the F distribution in multivariate analysis. *Annals of Mathematical Statistics, 29,* 885-891.

Globisch, J., Hamm, A. O., Esteves, F., & Öhman, A. (1994). Timecourse of the startle reflex modulation during short versus sustained visual stimulation in simple phobics and normals [Abstract]. *Psychophysiology, 31* (Suppl. 1), S90.

Graham, F. K. (1975). The more or less startling effects of weak prestimulation. *Psychophysiology, 12*, 238-248.

Goldsmith, H. H. & Rothbart, M. K. (1999). *The Laboratory Temperament Assessment Battery* (Prelocomotor Version, Edition 3.1). Madison, WI: University of Wisconsin-Madison.

Graham, F. K., Anthony, B. J., & Zeigler, B. L. (1983). The orienting response and developmental processes. In D. A. T. Siddle (Ed.), *Orienting and habituation: Perspectives in human research* (pp. 371-430). Exter, England: John Wiley.

Graham, F. K., & Clifton, R. K. (1966). Heart rate changes as the component of the orienting response. *Psychological Bulletin, 65*, 305-320.

Greenwald, M. K., Bradley, M. M., & Cuthbert, B. N., & Lang, P. J. (1990). The acoustic startle reflex indexes aversive learning [Abstract]. *Psychophysiology, 28,* S26.

Greenwald, M. K., Cook, E. W., & Lang, P. J. (1989). Affective judgment and psychophysiological response: Dimensional covariation in the evaluation of pictorial stimuli. *Journal of Psychophysiology, 3,* 51-64.

Grillon, C., Ameli, R., Woods, S. W., Merikangas, K., & Davis, M. (1991). Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology, 28,* 588-595.

Grillon, C., Ameli, R., Woods, S. W., Merikangas, K., & Davis, M. (1993). Measuring the time-course of anxiety using the fear-potentiated startle reflex. *Psychophysiology, 30,* 340-346.

Grillon, C., & Bass, J. (2003). A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clinical Neurophysiology, 114,* 1557-1579.

Grillon, C., Dierker, L., & Merikangas, K. R. (1997). Startle modulation in children at risk for anxiety disorders and/or alcoholism. *Journal of the American Academy of Child and Adolescent Psychiatry, 36,* 925-932.

Grillon, C., Merikangas, K. R., Dierker, L., Snidman, N., Arriaga, R. I., Kagan, J., Donzella, B., Dikel, T., & Nelson, C. (1999). Startle potentiation by threat of aversive stimuli and darkness in adolescents: a multi-site study. *International Journal of Psychophysiology, 32,* 63-73.

Hackley, S. A., & Graham, F. K. (1984). Early selective attention effects on cutaneous and acoustic blink reflexes. *Physiological Psychology, 11,* 235-242.

Hamm, A. O., Greenwald, M. K., Bradley, M. M., & Lang, P. J. (1993). Emotional learning, hedonic changes, and the startle probe. *Journal of Abnormal Psychology, 102,* 453-465.

Haviland, J. M., & Lelwica, M. (1987). The induced affect response: 10-week-old infants' responses to three emotion expressions. *Developmental Psychology, 23,* 97-104.

Hawk, L. W., & Cook, E. W., III (1997). Affective modulation of tactile startle. *Psychophysiology, 34,* 23-31.

Hilgard, E. R. (1933). Reinforcement and inhibition of eyelid reflexes. *Journal of General Psychology, 8,* 85-13.

Hirano, C., Russell, A. T., Ornitz, E. M., & Liu, M. (1996). Habituation of P300 and reflex motor (startle blink) responses to repetitive startling stimuli in children. *International Journal of Psychophysiology, 22,* 97-109.

Hitchcock, J. M., & Davis, M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. *Behavioral Neuroscience, 100,* 11-22.

Hitchcock, J. M., & Davis, M. (1987). Fear-potentiated startle using an auditory conditioned stimulus: Effect of lesions of the amygdala. *Physiology and Behavior, 39,* 403-408.

Hitchcock, J. M., & Davis, M. (1991). Efferent pathway of the amygdala involved in condiotioned fear as measured with the fear-potentiated startle paradigm. *Behavioral Neuroscience, 105,* 826-842.

Hunter, M. A., & Ames, E. W. (1988). A multifactor model of infant preferences for novel and familiar stimuli. In L. P. Lipsitt (Ed.), *Advances in child development and behavior* (pp. 69-95). New York: Academic.

Izard. C. E., Fantauzzo, C. A., Castle, J. M., Haynes, O. M., Rayias, M. F., & Putnam, P. H. (1995). The ontogeny and significance of infants' facial expressions in the first 9 months of life. *Developmental Psychology*, *31*, 997-1013.

Jackson, D. C., Malmstadt, J. R., Larson, C. L., & Davidson, R. J. (2000). Suppression and enhancement of emotional responses to unpleasant pictures. *Psychophysiology, 37,* 515-522.

Jansen, D. M., & Frijda, N. H. (1994). Modulation of the acoustic startle response by film-induced fear and sexual arousal. *Psychophysiology, 31,* 565-571.

Kagan, J. (1974). Discrepancy, temperament, and infant distress. In M. Lewis & L. Rosenblum (Eds.), *Origins of fear*. New York: Wiley.

Kagan, J., & Snidman, N. (1991). Temperamental factors in human development. *American Psychologist, 46,* 856-862.

Kaviani, H., Gray, J. A., Checkley, S. A., Kumari, V., & Wilson, G. D. (1999). Modulation of the acoustic startle reflex by emotionally-toned film-clips. *International Journal of Psychophysiology, 32*, 47-54.

Kaviani, H., Wilson, G. D., & Checkley, S. A. (1998). Modulatin of the human acoustic startle reflex by pleasant and unpleasant odors. *Journal of Psychophysiology, 12*, 353-361.

Kestenbaum, R., & Nelson, C. A. (1990). The recognition and categorization of upright and inverted emotional expressions by 7-month-old infants. *Infant Behavior and Development, 13,* 497-511.

Klorman, R., Cicchetti, D., Thatcher, J. E., Ison, J. R. (2003). Acoustic startle in maltreated children. *Journal of Abnormal Child Psychology*, *31*, 359-370.

Koch, M., Schmid, A., & Schitzler, H. (1995). Pleasure-attenuation of startle is disrupted by lesions of the nucleus accumbens. *NeuroReport, 7*, 1442-1446.

Konorski, J. (1967). *Integrative activity of the brain: An interdisciplinary approach.* Chicago: University of Chicago Press.

Kopp, C. B. (1989). Regulation of distress and negative emotions: A developmental view. *Developmental Psychology*, *25*, 343-354.

Kopp, C. B., & Neufeld, S. J. (2003). Emotional development during infancy. In R. J. Davidson, K. R. Scherer & H. H. Goldsmith (Eds.), *Handbook of affective sciences* (pp. 347-374). New York: OUP.

Kotsoni, E., de Haan, M., & Johnson, M. H. (2001). Categorical perception of facial expressions by 7-month-old infants. *Perception, 30*, 1115-1125.

LaBarbera, J. D., Izard, C. E., Vietze, P., & Parisi, S. A. (1976). Four- and six-month-old infants' visual responses to joy, anger, and neutral expressions. *Child Development, 47,* 535-538.

Lacey, B. C., & Lacey, J. I. (1974). Studies of heart rate and other bodily processes in sensorimotor behavior. In P. A. Obrist, A. H. Black., J. Brener., & L. V. Di Cara (Eds.), *Cardiovascular psychophysiology: Current issues in response mechanism, biofeedback and methodology* (pp. 538-564). Chicago, IL: Aldine.

Landis, C., & Hunt, W. A. (1939). *The startle pattern.* New York: Farrar & Rinehart.

Lang, P. J. (1995). The emotion probe: Studies of motivation and attention. *American Psychologist, 50,* 372-385.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1990). Emotion, attention, and the startle reflex. *Psychological Review, 97,* 377-395.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1992). A motivational analysis of emotion: Reflex-cortex connections. *Psychological Science, 3,* 44-49.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1997a). International Affective Picture System (IAPS): Technical manual and affective ratings. NIMH Center for the Study of Emotion and Attention.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1997b). Motivated attention: Affect, activation, and action. In P. J. Lang, R. F. Simons, & M. T. Balaban (Eds.), *Attention and orienting: Sensory and motivational processes* (97-136). Hillsdale, NJ: Erlbaum.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1998). Emotion, motivation, and anxiety: Brian mechanism and psychophysiology. *Biological Psychiatry, 44*, 1248-1263.

Lang, P. J., Bradley, M. M., Drobes, D. J., & Cuthbert, B. N. (1995). Emotional perception: Fearful beasts, scary people, sex, sports, disgust, and disasters [Abstract]. *Psychophysiology, 32* (Suppl. 1), S48.

Lang, P. J., Greenwald, M. K., Bradley, M. M., & Hamm, A. O. (1993). Looking at pictures: Affective, facial, visceral, and behavioral reactions. *Psychophysiology, 30,* 261-273.

Larson, C. L., Ruffalo, D., Nieter, J. Y., & Davidson, R. J. (2000). Temporal stability of the emotion-modulated startle response. *Psychophysiology, 37,* 92-101.

LeBar, K. S., LeDoux, J. E., Spencer, D. D., & Phelps, E. A. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. *Journal of Neuroscience, 15,* 6846-6855.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus, sensory interface of the amygdala in fear conditioning. *Journal of Neuroscience, 10,* 1062-1069.

Lewis, M. (2000). The emergence of human emotions. In M. Lewis & J. M. Haviland-Jones (Eds.), *Handbook of emotions* (pp. 265-280). New York: Guilford.

Lipp, O. V., Sheridan, J., & Siddle, D. A. T. (1994). Human blink startle during aversive and non-aversive Pavlovian conditioning. *Journal of Experimental Psychology, 20,* 380-389.

Ludemann, P. M. (1991). Generalized discrimination of positive facial expressions by seven- and ten-month-old infants. *Child Development, 62*, 55-67.

Ludemann, P. M., & Nelson, C. A. (1988). Categorical representation of facial expressions by 7-month-old infants. *Developmental Psychology, 24,* 492-501.

Malatesta, C. Z., Grigoryev, P., Lamb, C., Albin, M., & Culver, C. (1986). Emotion socialization and expressive development in preterm and full-term infants. *Child Development, 57*, 316-330.

Malatesta, C. Z., & Haviland, J. M. (1982). Learning display rules: the socialization of emotion expression in infancy. *Child Development, 53*, 991-1003.

Manber, R., Allen, J. J. B., Burton, K., & Kaszniak, A. W. (2000). Valence-dependent modulation of psychophysiological measures: Is there consistency across repeated testing? *Psychophysiology, 37*, 683-692.

McManis, M. H., Bradley, M. M., Cuthbert, B., & Lang, P. J. (1995). Kids have feelings too: Children's physiological response to affective pictures [Abstract]. *Psychophysiology, 32* (Suppl. 1), S53.

Miltner, W., Matjak, M., Braun, C., Diekmann, H., & Brody, S. (1994). Emotional qualities of odors and their influence on the startle reflex in humans. *Psychophysiology, 31,* 107-110.

Nelson, C. (1987). The recognition of facial expressions in the first two years of life: Mechanisms of development. *Child Development, 58,* 889-909.

Nelson, C. A., & Dolgin, K. (1985). The generalized discrimination of facial expressions by 7-month-old infants. *Child Development, 56*, 58-61.

Ornitz, E. M. (1999). Startle modification in children and developmental effects. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification:*

Implications for neuroscience, cognitive science, and clinical science (pp. 245-266). New York, NY: Cambridge University Press.

Ornitz, E. M., Russell, A. T., Yuan, H., & Liu, M. (1996). Autonomic, electroencephalographic, and myogenic activity accompanying startle and its habituation during mid-childhood. *Psychophysiology, 33*, 507-513.

Oster, H., & Ewy, R. (1980). *Discrimination of sad vs happy faces by 4-month-olds: When is a smile seen as a smile?* Unpublished manuscript, University of Pennsylvania.

Oster, H., Hegley, D., & Nagel, L. (1992). Adult judgments and the fine-grained analysis of infant facial expressions: Testing the validity of a priori coding formulas. *Developmental Psychology, 28*, 1115-1131.

Paradiso, S., Johnson, D. L., Andreasen, N. C., O'Leary, D. S., Watkins, G. L., Ponto, L. L., & Hichwa, R. D. (1999). Cerebral blood flow changes associated with attribution of emotional valence to pleasant, unpleasant, and neutral visual stimuli in a PET study of normal subjects. *American Journal of Psychiatry, 156*, 1618-1629.

Phelps, E. A., O'Connor, K. J., Gatenby, J. C., Gore, J. C., Grillon, C., & Davis, M. (2001). Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience, 4,* 437-441.

Pitkänen, A., Stefanacci, L., Farb, C. R., Go, C.-G., LeDoux, J. E., & Amaral, D. G. (1995). Intrinsic connections of the rat amygdaloid complex: Projections originating in the lateral nucleus. *Journal of Comparative Neurology, 356,* 288-310.

Porges, S. W. (1992). Autonomic regulation and attention. In B. Campbell, H. Hayne, & R. Richardson (Eds.), *Attention and information processing in infants and adults* (pp. 201-223). Hillsdale, NJ: Erlbaum.

Putnam, L. E., & Vanman, E. J. (1999). Long lead interval startle modification. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (pp. 72-92). New York, NY: Cambridge University Press.

Richards, J. E. (1993). Infant blink reflexes as a function of visual attention status. *Psychophysiology, 15,* 377-379.

Richards, J. E. (2000). Development of multimodal attention in young infants: Modification of the startle reflex by attention. *Psychophysiology, 37,* 65-75.

Roder, B. J., Bushnell, E. W., & Sasseville, A. M. (2000). Infants' preferences for familiarity and novelty during the course of visual processing. *Infancy, 1*, 491-507.

Rosen, J. B., Hitchcock, J. M., Sananes, C. B., Miserendino, M. J. D., & Davis, M. (1991). A direct projection from the central nucleus of the amygdala to the acoustic startle pathway: Anterograde and retrograde tracing studies. *Behavioral Neuroscience, 105,* 817-825.

Sabatinelli, D., Bradley, M. M., & Lang, P. J. (2001). Affective startle modulation in anticipation and perception. *Psychophysiology, 38,* 719-722.

Schmid, A., Koch, M., & Schitzler, H. U. (1995). Conditioned pleasure attenuates the startle response in rats. *Neurobiology of Learning & Memory, 64*, 1-3.

Schmidt, L. A., & Fox, N. A. (1998a). Fear-potentiated startle responses in temperamentally different human infants. *Developmental Psychobiology, 32,* 113-120.

Schmidt, L. A., & Fox, N. A. (1998b). Acoustic startle electromyographic (EMG) activity indexed from an electroculographic (EOG) electrode placement: A methodological note. *International Journal of Neuroscience, 93*, 185-188.

Schneirla, T. (1959). An evolutionary and developmental theory of biphasic processes underlying approach and withdrawal. In M. Jones (Ed.), *Nebraska symposium on motivation* (pp. 1-42). Lincoln: University of Nebraska Press.

Schupp, H. T., Cuthbert, B. N., Bradley, M. M., Birbaumer, N., & Lang, P. J. (1997). Probe P3 and blinks: Two measures of affective startle modulation. *Psychophysiology, 34,* 1-6.

Schupp, H. T., Cuthbert, B. N., Bradley, M. M., Lang, P. J., & Birbaumer, N. (1993). Imagining the percept: Cortical and peripheral measures [Abstract]. *Psychophysiology, 30,* S58.

Schwartz, G. M., Izard, C. E., & Ansul, S. E. (1985). The 5-month-old's ability to discriminate facial expressions of emotion. *Infant Behavior and Development, 8*, 65-77.

Serrano, J. M., Iglesias, J., & Loeches, A. (1992). Visual discrimination and recognition of facial expressions of anger, fear, and surprise in four- to six-month-old infants. *Developmental Psychobiology, 25*, 411-425.

Serrano, J. M., Iglesias, J., & Loeches, A. (1995). Infants' responses to adult static facial expressions. *Infant Behavior and Development, 18*, 477-482.

Silverstein, L. D., Graham, F. K., & Bohlin, G. (1981). Selective attention effects on the reflex blink. *Psychophysiology, 18*, 240-247.

Simons, R. F., & Zelson, M. F. (1985). Engaging visual stimuli and reflex blink modification. *Psychophysiology, 22,* 44-49.

Soken, N. H., & Pick, A. D. (1999). Infants' perception of dynamic affective expressions: Do infants distinguish specific expressions? *Child Development, 70,* 1275-1282.

Spence, E., & Lang, P. J. (1990). Reading affective text: The startle probe response [Abstract]. *Psychophysiology, 27,* S65.

Sroufe, L. A., Waters, E., & Matas, L. (1974). Contextual determinants of infants affective response. In M. Lewis & L. Rosenblum (Eds.), *The origins of fear*. New York: Wiley.

Stern, R. M., Ray, W. J., & Quigley, K. S. (2001). *Psychophysiological recording.* New York: Oxford University Press.

Stifter, C. A., & Braungart, J. M. (1995). The regulation of negative reactivity in infancy: Function and development. *Developmental Psychology*, *31*, 448-455.

Sutton, S. K., Davidson, R. J., Donzella, B., Irwin, W., & Dottl, D. A. (1997). Manipulating affective state using extended picture presentations. *Psychophysiology, 34*, 217-226.

Tottenham, N., Borscheid, A., Ellertsen, K., Marcus, D.J., & Nelson, C.A. (April, 2002). *Categorization of Facial Expressions in Children and Adults: Establishing a Larger Stimulus Set*. Poster presented at the Cognitive Neuroscience Society annual meeting, San Francisco.

Tronick, E. Z., Ricks, M., & Cohn, J. F. (1982). Maternal and infant affective exchange: Patterns of adaptation. In T. Field & A. Fogel (Eds.), *Emotion and early interation* (pp. 83-100). Hillsdale, NJ: Erlbaum.

Ursin, H., Jellestad, F., & Cabrera, I. G. (1981). The amygdala, exploration and fear. In Y. Ben-Ari (Ed.), *The amygdaloid complex* (pp. 317-329). Amsterdam: Elesvier/North-Holland.

Van Boxtel, A., Boelhouwer, A. J. W., & Bos, A. R. (1998). Optimal EMG signal bandwidth and interelectrode distance for the recording of acoustic, electrocutaneous and photic blink reflexes. *Psychophysiology, 35,* 690-697.

Vecchierini-Blineau, M. F., & Guiheneue, P. (1984). Maturation of the blink reflex in infants. *European Neurology, 23,* 449-458.

Vrana, S. R., Spence, E. L., & Lang, P. J. (1988). The startle probe response: A new measure of emotion? *Journal of Abnormal Psychology, 97,* 487-491.

Waters, A. M., Lipp, O. V., & Cobham, V. E. (2000). Investigation of threat-related attentional bias in anxious children using the startle eyeblink modification paradigm. *Journal of Psychophysiology, 14,* 142-150.

Winton, W. M., Putnam, L. E., & Krauss, R. M. (1984). Facial and autonomic manifestations of the dimensional structure of emotion. *Journal of Experimental Social Psychology, 20,* 195-216.

Yerkes, R. M. (1905). The sense of hearing in frogs. *Journal of Comparative Neurology and Psychology, 15,* 279-304.